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ANTICONVULSANT DRUGS : THEIR THERAPEUTIC
AND BIOCHEMICAL EFFECTS IN EPILEPTIC PATIENTS

by

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A thesis submitted for the degree of Doctor of
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SUMMARY

Satisfactory seizure control in epileptic patients may be difficult to achieve when anticonvulsant drug dosage is adjusted empirically. This may be due, in part, to individual variations in metabolism or distribution. Section 1 of this thesis includes studies of how serum concentrations of anticonvulsants may vary in different patients and the relationship between serum concentrations and therapeutic effects.

As shown in Chapter 3, serum concentrations of phenytoin show wide variations between different patients. Chapter 4 describes a study to investigate the value of a nomogram (Richens and Dunlop, 1975) in adjusting serum concentrations. Control of major seizures, but not minor seizures, improved when the serum concentrations were raised into the postulated therapeutic range in patients on multiple drug therapy. The nomogram was found to be of only limited value in predicting serum concentrations, apparently because the phenobarbitone that these patients were receiving inhibits phenytoin metabolism and may alter the relationship between dose and serum concentration.

The interactions between phenytoin and phenobarbitone are complex since, as shown in Chapter 3, administration of phenytoin inhibits phenobarbitone metabolism and increases phenobarbitone serum concentrations in patients receiving phenobarbitone as the drug itself or as a metabolic product of primidone. In addition, in the study described in Chapter 5, phenytoin was found to lower serum concentrations of primidone by inducing its conversion to phenylethylmalonamide (PEMA). The relationship between primidone dose and serum concentration is non-linear, apparently due to induction by primidone of its own metabolism.

Serum concentrations and therapeutic effect of a new benzodiazepine anticonvulsant, clonazepam, are described in Chapter 6. Improvement in seizure control was greater and side-effects were less in patients who were receiving

clonazepam alone or low doses of other drugs. There was no effect of clonazepam on serum concentrations of other anticonvulsants, but administration of other anticonvulsants appeared to induce the metabolism of clonazepam, possibly to a toxic derivative.

A comparative trial of clonazepam against another recently introduced anticonvulsant, sodium valproate, is reported in Chapter 7. Neither drug significantly reduced the frequency of major seizures but sodium valproate therapy significantly improved control of minor seizures. Sodium valproate administration produced an increase in phenobarbitone serum concentrations in some patients and in this trial phenobarbitone doses were reduced where necessary to ensure that the therapeutic action of sodium valproate was not mediated via this effect. Serum concentrations of sodium valproate were similar in different patients and there was no apparent effect of administration of other drugs.

In Chapter 7 it is also shown that sodium^{valproate} administration reduces serum concentrations of phenytoin and it is suggested that this may be due to displacement of phenytoin from its plasma protein binding sites. Tissue concentrations of drugs depend on the free concentrations in serum and studies of protein binding of phenytoin and other anticonvulsants was described in Section 2 of this Thesis. In Chapter 8 it is shown that protein binding of phenytoin may vary significantly between different patients and Chapters 9 and 10 describe methods which may be used to measure protein binding routinely in epileptic patients. Saliva concentrations of phenytoin were measured by a new radioimmunoassay technique and are shown to be dependent on the free concentration in serum. In addition, measurement of phenytoin in red blood cells may be a useful index of protein binding.

Section 3 of this Thesis includes studies of some of the biochemical effects of anticonvulsants. Serum and CSF concentrations of folate are lowered by anticonvulsant therapy and in Chapter 11 a trial is reported of the effects of administration of folate either as folic acid or 5-formyltetrahydrofolate to epileptic patients. Both groups

of patients showed increases in serum and CSF concentrations of folate but there were no significant effects on brain amine metabolism, as measured by CSF concentrations of HVA and HIAA, or on seizure control. There was, however, a significant fall in serum concentrations of phenytoin and this may account for an increase in major seizures found in some patients in this study and in studies by other workers.

The effects of anticonvulsants on liver enzyme induction were studied in Chapter 12 by measuring serum levels of γ -glutamyl-transpeptidase. The metabolism of primidone, clonazepam and folate were found to be related to the combined doses of enzyme-inducing drugs. No evidence was, however, found between enzyme induction and serum concentrations of calcium or triglyceride. It is also possible, as described in Chapter 13, that the effect of phenytoin in lowering serum concentrations of IgA may be explained by an induction of its breakdown but it is suggested that the low folate concentrations in patients on anticonvulsant therapy may antagonise this effect.

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ABBREVIATIONS

DPH	diphenylhydantoin (phenytoin)
PB	phenobarbitone
PEMA	phenylethylmalonamide
HPPH	p-hydroxy-phenytoin
CSF	cerebrospinal fluid
GLC	gas liquid chromatography
5-HIAA	5-hydroxy-3-indole acetic acid
HVA	homovanillic acid
γ GT	γ -glutamyl transpeptidase
Ig	immunoglobulin
EEG	electroencephalogram
TMAH	trimethylammonium hydroxide
TMPAH	trimethylphenylammonium hydroxide
RBC	red blood cell
SD	standard deviation
r	regression coefficient
p	probability

UNITS USED

sec	second
kg	kilogram 1×10^3 gram
mg	milligram 1×10^{-3} gram
μ g	microgram 1×10^{-6} gram
ml	millilitre 1×10^{-3} litre
μ l	microlitre 1×10^{-6} litre
μ U/l	micro Units per litre 1×10^{-6} Units per litre
mmol/l	millimoles per litre 1×10^{-3} moles per litre
μ mol/l	micromoles per litre 1×10^{-6} moles per litre
nmol/l	nanomoles per litre 1×10^{-9} moles per litre
$^{\circ}$ C	degrees centigrade

CHAPTER 1

INTRODUCTION

Historically, epilepsy is a term which was introduced by the Ancient Greeks to describe the phenomenon in which a man was apparently "seized" by mysterious forces. Hippocrates (460-357 BC) suggested that epilepsy was due to a disturbance of the brain, and in the seventeenth century Willis attributed epileptic fits to an explosion of animal spirits within the brain. Scientific understanding of epilepsy did not begin until the nineteenth century however. In 1870, the experiments of Trites and Hitzig showed that electrical stimulation of the dog's cortex produced fits and in the same year Hughlings Jackson suggested that epilepsy was due to occasional discharges of neurones, beginning at and spreading from a focus in the brain. The introduction of electroencephalography (Berger, 1929) permitted the electrical recording of the discharge and has been a major tool in the study of seizures.

Epilepsy is among the most common of chronic neurological disorders, the incidence being reported as 1 in 200 of the population (Lennox, 1960). In 75% of the patients it begins before the age of 20 and in two thirds of all patients it is a chronic recurring problem despite drug therapy which must, therefore, be lifelong (Rodin, 1968). The aetiological factors in epilepsy have not been precisely established, but it is known that damage to the brain such as produced by infections (meningitis, encephalitis), cerebral injuries sustained in association with birth or from other causes and metabolic disorders (hypoglycaemia, hypernatraemia) predispose some individuals to the development of the disease.

Epileptic attacks differ widely in their clinical presentation. There may be only a momentary suspension of activity, as in a petit mal attack. In others, complex alterations of behaviour characterise the attack. Consciousness is usually, but not always, affected; some seizures consist of nothing

more than an altered sensation. The manifestations of the seizures depend upon several factors, foremost among which are the site of origin of the epileptic discharge and the pattern of spread of the discharge within the brain. The most generally accepted classification of seizures is that proposed by the International League against Epilepsy (Gastaut, 1970) which is shown in Table 1.1.

Seizures may be divided into two groups: those that begin as local processes in relatively restricted populations of neurones are described as focal or partial seizures; and those that are primarily generalised, having a diffuse and unspecific influence upon cerebral function. Secondary generalisation also occurs, when this same diffuse system is activated by spread from an epileptic discharge that is initiated in a focus.

The tonic-clonic or grand mal attack is a generalised seizure and is the type of attack most likely to be equated with epilepsy by the layman. The patient loses consciousness and the sequence of motor events usually proceeds from tonic muscular stiffening to clonic fits. The body initially goes rigid and the patient falls, this being followed by a period of symmetrical jerking and relaxation of the extremities. By contrast, the absence attack is a generalised seizure in which the main characteristic is simply a brief suspension of consciousness and may be so mild as to make recognition difficult. Myoclonic seizures which may occur singly or repetitively, are sudden brief muscular contractions.

The nature of the clinical symptoms in a partial seizure depends to a great extent upon the functional importance of the area of the brain from which the discharge emanates. Usually such foci are in the cerebral cortex. Partial seizures may be separated into two major categories; those with elemental and those with complex symptoms. Seizures with complex symptomatology, including psychomotor attacks, characteristically arise from temporal lobe foci on one or both sides. The psychomotor attack (or automatism) is a seizure involving a period of altered behaviour for which

1. Partial Seizures (local onset)
 - A. With elementary symptomatology - generally without impaired consciousness
 1. With motor symptoms (includes Jacksonian seizures)
 2. With special sensory or somatosensory symptoms
 3. With autonomic symptoms
 4. Compound forms
 - B. Partial seizures with complex symptomatology - generally with impairment of consciousness
 1. Impaired consciousness only
 2. Cognitive symptomatology
 3. Affective symptomatology
 4. Psychosensory symptomatology
 5. Psychomotor symptomatology (automatisms)
 6. Compound forms
 - C. Partial seizures secondarily generalised - usually tonic-clonic in type
2. Generalised Seizures (bilaterally symmetrical without local onset)
 1. Absences (including petit mal)
 2. Bilateral massive epileptic myoclonus
 3. Infantile spasms
 4. Clonic seizures
 5. Tonic seizures
 6. Tonic-clonic seizures (grand mal)
 7. Atonic seizures
 8. Akinetic seizures
3. Unilateral Seizures
4. Unclassified Seizures

Table 1.1 International Classification of Epileptic Seizures (1970)

the patient is amnesic and during which he appears to respond in a limited fashion to his environment. Partial seizures with elementary symptoms include motor seizures with characteristic movements (including Jacksonian seizures) or with inhibition of movement, and various forms of sensory seizures, in which the patient experiences somatic, visual, auditory and vertiginous symptoms.

The first effective drug treatment came in 1857, when Locock introduced bromides, on the erroneous supposition that epilepsy was caused by sexual overactivity and would thus be relieved by an anti-aphrodisiac. Prior to this time, treatment of epilepsy was generally bizarre and included trephination of the skull to let the evil spirits escape or, in Roman times, drinking the fresh blood of a gladiator. Bromide salts remained the only successful treatment until, with the introduction of phenobarbitone by Hauptman in 1912, a truly effective anticonvulsant therapy arrived. This was followed by the introduction of diphenylhydantoin by Merritt and Putman in 1938, and a wide range of anticonvulsants is now available (Table 1.2). Most patients are, however, still treated with combinations of phenytoin, phenobarbitone and primidone. Primidone is, in fact, converted in the body partly to phenobarbitone (Bogue, 1956) and this may be the basis of its anticonvulsant action.

The use of these drugs is empirically based and the mechanisms of their actions remain little understood. The primary action of phenytoin appears to be in stabilising excitable membranes, possibly due to a decrease in intracellular sodium and an increase in the extracellular to intracellular Na^+ (Woodbury, 1955). Phenobarbitone is a sedative and has a depressing effect on synaptic activity. It may also act via a blocking effect on cation transport through the cellular membrane, with a resulting decrease in the Na^+ and K^+ fluxes which accompany excitation and inhibition (Bunker and Vandam, 1965). Phenytoin, phenobarbitone and primidone are all wide spectrum anticonvulsants and are effective against most types of seizures, although only minimally so in petit mal which is usually

GENERIC NAME	TRADE NAME
A. BARBITURATES	
1. Phenobarbitone	Gardenal
2. Primidone	Mysoline
B. HYDANTOINATES	
1. Diphenylhydantoin	Epanutin
2. Methoin	Mesontoin
3. Ethotoin	Peganone
C. OXAZOLIDINIDIONES	
1. Trimethadione	Tridione
2. Paramethadione	Paradione
D. SUCCINIMIDES	
1. Ethosuximide	Zarontin
2. Phensuximide	Milontin
3. Methosuximide	Cilontin
E. BENZODIAZEPINES	
1. Diazepam	Valium
2. Nitrazepam	Mogadon
3. Clonazepam	Rivotril
F. MISCELLANEOUS	
1. Carbamazepine	Tegretol
2. Sodium Valproate	Epilim
3. Sulthiame	Ospolot
4. Pheneturide	Benuride

Table 1.2 Some Currently Available Anticonvulsants

treated with ethosuximide.

The ideal anticonvulsant drug should be capable of complete suppression of all types of seizure at a dosage level that does not cause sedation or other toxic effects. No such drug exists and, with the aim of maximising seizure control, with the minimum of toxic effects, it has become standard practice to utilise multiple drug therapy in the management of epilepsy. It is, therefore, important to know if one drug will alter the action of another where they are taken together.

Drug interactions may occur as a result of alteration of the absorption, distribution or elimination of one drug by another, or from combination of their actions or effects. In particular, interactions involving anticonvulsants may involve a rise or fall of the serum concentration of the primary drug, after addition of a second agent. In order for a drug to act it must reach the site of its action (receptor), presumably the central nervous system in the case of anticonvulsants. The concentration of drug that reaches receptor, and hence the effect, depends upon the serum level. It would be expected that measurement of serum concentration will give a more accurate guide to the therapeutic or toxic effects of a drug than will the daily dose. Improved techniques for the assay of drugs, particularly using gas chromatography, have made their routine measurement possible and the value of determining serum concentrations of anticonvulsant drugs in the clinical management of patients with epilepsy has been emphasised in numerous publications (reviewed in Woodbury et al, 1972; Eadie and Tyrer, 1974; Kutt and Penry, 1974).

The aim of Section 1 of this thesis is to study further the value of the measurement of serum anticonvulsant concentrations in the treatment of epileptic patients and how various factors, particularly the administration of other drugs, affect the relationship between dose and serum concentration. After a review of methods used for the measurement of anticonvulsant drugs in Chapter 2, a survey is described in Chapter 3 of an inpatient and an outpatient

epileptic population which had the aim of investigating what drugs these patients were receiving and how well patients were managed without the availability of information on drug levels. It was found that patients on phenytoin, which is the most commonly used anticonvulsant, showed serum concentrations below the commonly accepted therapeutic range; a study was therefore designed and is described in Chapter 4 to investigate whether increasing serum concentrations into the therapeutic range improves seizure control. In Chapter 3 it is also shown that serum concentrations of phenytoin may increase with age; the possible effects of age on phenytoin metabolism are examined in Chapter 4A.

Many patients receive combined therapy of phenytoin with phenobarbitone or primidone, and interactions between these drugs may be of major therapeutic importance. Chapter 3 includes an investigation of the possible effects of phenobarbitone or primidone on serum concentrations of phenytoin and the possible importance of these interactions is examined further in Chapter 4. Conversely, phenytoin administration may affect serum concentrations of phenobarbitone and studies in Chapters 3 and 4 show that phenytoin may inhibit phenobarbitone metabolism. The effects of phenytoin on primidone metabolism are more complex and appear to involve both inhibition and induction effects; this is described in Chapter 5.

The use of information on serum drug levels may be particularly valuable in the evaluation of new drugs. Chapters 6 and 7 describe trials of two newly introduced anticonvulsants, clonazepam and sodium valproate, which were designed to study the possibility of drug interactions and define therapeutic ranges of serum concentrations for these drugs.

Tissue concentrations of drugs depend on the free rather than the total concentration in plasma. Drugs such as phenytoin may be highly bound to proteins in plasma, and variations in protein binding may affect the therapeutic response. Section 2 of this thesis is concerned with the study of protein binding of anticonvulsants. In Chapter 8

the possible effects of various other factors (including plasma albumin concentration, administration of other drugs and the effect of temperature) on protein binding of phenytoin are examined, and the protein binding is shown to vary significantly between different patients. However, standard methods for measuring protein binding of drugs are generally too tedious to be used routinely in the clinical management of epileptic patients; Chapters 9 and 10 are concerned with the evaluation of simple new methods for studying binding. In Chapter 9 the concentration of phenytoin in saliva is shown to be equivalent to the free concentration in plasma and Chapter 10 describes how the uptake of phenytoin and other anticonvulsants by red blood cells is related to the free concentration of plasma.

The study of the pharmacokinetics of anticonvulsant drugs is important not only because it offers a means of improving seizure control, but also because it offers useful insights into the mechanisms of some of the biochemical effects of anticonvulsants. Section 3 of this thesis is concerned with an examination of the relationship of some of the biochemical effects of anticonvulsants to their serum concentrations and to their effects on the drug-metabolising system in the liver. Chapter 11 is concerned with a study of the reduced folate levels found in patients on anticonvulsant therapy and offers an interesting example of a link between a biochemical effect of a drug and its metabolism - it is found that administration of folate may decrease phenytoin serum concentrations, presumably due to increased metabolism of the drug, but conversely it is suggested that the effect of phenytoin and other anticonvulsants in reducing serum folate concentrations may be due to induction of folate metabolism. Evidence that folate metabolism may be induced by anticonvulsants is described in Chapter 12, there being a significant relationship between serum folate and levels of γ -glutamyltranspeptidase (γ GT). The action of anticonvulsant drugs in raising γ GT levels has been used as an index of their ability to induce activity of the drug-metabolising system in the liver and a method is described in Chapter 12 whereby the potential enzyme-inducing effect of a given drug regime can be

quantified in terms of 'Drug Units'. The relationship is described between the Drug Units that a patient is receiving and the metabolism of clonazepam and primidone and the possible effects of anticonvulsants on calcium metabolism, which may cause osteomalacia, are investigated. In Chapter 13 it is suggested that the low serum IgA levels obtained in patients on anticonvulsant therapy may be due to induction of breakdown of the protein; in addition, it is suggested that the lowered folate concentrations antagonise this effect and that folate therapy also increases immunoglobulin catabolism.

SUMMARY

1. The history of the understanding of epilepsy and the nature of the disease are briefly described. The types of epileptic seizure and the drugs available for treatment are tabulated.
2. The reasons for embarking on this study are given along with an outline of the structure of the thesis.

CHAPTER 2

MEASUREMENT OF ANTICONVULSANT DRUGS

The major part of the work described in this thesis involves measurement of anticonvulsant drugs, either in serum or in other biological fluids. In this Chapter the techniques available for the assay of anticonvulsant drugs are reviewed and the methods used for the studies in this thesis are summarised. Details of these methods are included in the appendix.

Techniques for Measuring Anticonvulsant Drugs

A variety of methods for measuring concentrations of phenytoin and other drugs have been developed over the last 20 years. The major methods that have been used can be divided into six basic types :

1. Spectrophotometric techniques such as that of Olesen, 1967. These show a lack of specificity and sensitivity and tend to give unreliable results (Richens, 1975).
2. Thin-layer chromatography techniques. That developed by Pippenger (1969) allows the measurement of approximate levels of phenytoin, phenobarbitone, primidone and phenylethylmalonamide (PEMA) and such techniques may be useful for routine screening of large numbers of specimens.
3. Gas-liquid chromatographic (GLC) techniques, in which the drug may be chromatographed directly or a derivative is formed.
4. High-pressure chromatographic methods such as that of Adams and Vandemark (1976) which allows the simultaneous determination of phenytoin, phenobarbitone, primidone, carbamazepine and ethosuximide. These methods provide good sensitivity without the derivatisation that may be necessary in GLC techniques, but require equipment that is not readily available in most laboratories.

5. Radioimmunoassay. This is a particularly sensitive technique and is useful for assaying drugs such as clonazepam whose concentrations in serum are low or for assaying drugs in small volumes of biological fluids such as saliva. However, there may be difficulties in obtaining a specific antibody; in the radioimmunoassay for clonazepam other benzodiazepines interfere. Only one drug may be assayed at a time and the cost involved is likely to be greater than with gas chromatography.
6. Enzyme immunoassay. This is an analogous method to radioimmunoassay and also suffers from the disadvantage that it is expensive to use. Also, it cannot be readily adapted to assay new drugs unless antibodies against these drugs are available. However, it is a particularly simple technique to use.

Gas Chromatography of Anticonvulsant Drugs

The generally most useful technique for measurement of anticonvulsants appears to be GLC. This can be used to measure most anticonvulsants, often several simultaneously, uses equipment which is readily available in most laboratories, and provides reliable and accurate results (Richens, 1975). The methods which have been developed are summarised in the accompanying table (Table 2.1).

Some workers chromatograph the unchanged drugs (Pippinger and Gillen, 1969; Evenson et al, 1970; Van Meter, 1970; Gardner-Thorpe et al, 1972; Toseland et al, 1972), while others form derivatives to reduce polarity and subsequent absorption and peak tailing. The commonest approach is the formation of 1,3-dimethyl derivatives of the drugs. Methylation prior to chromatography (Sandberg et al, 1968) increases the number of manipulative steps following extraction. On-column methylation with tetramethylammonium hydroxide (TMAH) (McGee, 1970) or trimethylphenylammonium hydroxide (TMPAH) (Hammer et al, 1970; Kupferberg, 1970; Goudie and Burnett, 1972; Kananen et al, 1972; Perchalski et al, 1973;

YEAR	AUTHOR	ANTICONVULSANTS ASSAYED	DERIVATISATION
1968	Sandberg et al	DPH	Methylation before chromatography
1969	Pippinger and Gillen	DPH, PB, Primidone	-
1970	Chang and Glazco	DPH, HPPH	Trimethylsilyl derivative
1970	Evenson et al	DPH, Primidone	-
1970	Kupferberg	DPH, PB, Primidone	Methylation (TMPAH)
1970	MacGee	DPH	Methylation (TMAH)
1970	Van Meter	DPH, PB, Primidone	-
1971	Hammer et al	DPH, HPPH	Methylation (TMPAH)
1972	Baumel et al	DPH, Primidone, PEMA	Trimethylsilyl derivative
1972	Gardner-Thorpe et al	DPH, PB, Primidone, Carbamazepine	-
1972	Goudie and Burnett	DPH, PB, Primidone	Methylation (TMPAH)
1972	Kananen et al	DPH, PB, Primidone	Methylation (TMPAH)
1972	Toslandet al	DPH, PB, Primidone, Carbamazepine	-
1973	Perchalski et al	DPH, PB, Primidone	Methylation (TMPAH)
1973	Roger et al	DPH, PB, Primidone, Carbamazepine	Methylation (TMPAH)
1975	Kumps and Mardens	DPH, PB	Methylation (TMPAH)
1975	Friel and Troupin	DPH, PB, Primidone	Methylation (TMPAH)
1975	Hooper et al	PB	Ethylation
1975	Joern	DPH, PB, Primidone	Ethylation
1975	Least et al	DPH, Primidone, PEMA, Carbamazepine	Butylation
1976	Giovanniello and Pecci	DPH, PB	Methylation (TMPAH)
1976	Midha et al	DPH, HPPH	Trimethylsilyl derivative
1976	Nishina et al	DPH, PB, Primidone, Carbamazepine	Hexylation
			Methylation (TMPAH)
			Methylation (TMPAH)

Table 2.1 Gas Chromatographic methods for the Determination of
Phenytoin (DPH), Phenobarbitone (PB), Primidone,
p-hydroxy-phenytoin (HPPH), Phenylethylmalonamide (PEMA)
and Carbamazepine

Roger et al, 1973; Midha et al, 1976; Nishina et al, 1976) provides a simple technique; the latter reagent appears to give the better quantitative performance. Results of a quality control scheme (Richens, 1975) suggested that GLC with methylation was more accurate, at least for assay of phenytoin, than GLC without derivatisation. Other methods utilise trimethylsilyl (Chang and Glazco, 1970; Baumel et al, 1972; Least et al, 1975), ethylated (Kumps and Mardens, 1974; Friel and Troupin, 1975) hexylated (Giovanniello and Pecci, 1976) and butylated (Hooper et al, 1975) derivatives.

Since most epileptic patients receive various combinations of phenytoin, phenobarbitone and primidone, it is desirable that any routine method for measurement of serum levels should measure these drugs simultaneously. In the present study it was intended to investigate the metabolism of phenytoin and primidone and it was desirable that the method used could be adapted as easily as possible to the measurement of HPPH (in urine) and PEMA (in serum and urine). Also, a significant number of patients receive carbamazepine and ideally the method should also be capable of measuring this drug. However, there is no single method available which is capable of measuring phenytoin, phenobarbitone, primidone, carbamazepine, HPPH and PEMA. A method using on-column methylation with TMPAH has been described for the simultaneous measurement of phenytoin, phenobarbitone, primidone and carbamazepine (Roger et al, 1973), but the present author did not find that a reproducible carbamazepine peak was obtained. Carbamazepine is measured as a decomposition product but it was found that under the conditions used (without automatic sampler as in the original publication) the degree of decomposition was variable, and this has been confirmed by other workers.

A method in which the trimethylsilyl derivatives are chromatographed has been suggested for the simultaneous measurement of phenytoin, primidone, PEMA and carbamazepine (Least et al, 1975). However, the trimethylsilyl

derivative of phenobarbitone is unstable and this drug cannot be assayed by this procedure. Routine use of this method would require a separate determination of phenobarbitone, by methylation, in all patients receiving this drug or primidone. Thus in this study the routine method used for analysis of phenytoin, phenobarbitone and primidone involved methylation with TMPAH; the method was extended to include simultaneous measurement of HPPH in urine samples. Since this method could not be used for the assay of PEMA, this compound was determined separately as its trimethylsilyl derivative.

Extraction of Anticonvulsant Drugs

A considerable number of methods for extraction of drugs from serum have been used by different investigators, but they can be divided into two basic types : those in which the drugs are extracted into organic solvent and the solvent then evaporated; and those in which the methylating (or ethylating) agent is added to the solvent to extract the drugs so that no solvent evaporation is necessary. The latter technique offers advantages in terms of rapidity but has disadvantages in that the methylating agent used must be TMAH rather than TMPAH and the extracting solvent generally incorporates ether (Perchalski et al, 1973; Friel and Troupin, 1975; Joern, 1975), which is relatively dangerous to handle. Also, this method is not suitable for the extraction of PEMA. Therefore, the method used for extraction of drugs in this study was one in which the solvent was evaporated after extraction; before evaporation it was possible to split the extract into two parts - one for the assay of PEMA and one for the assay of phenytoin, phenobarbitone, primidone and PEMA. It would also be possible to combine the assay of PEMA, as its trimethylsilyl derivative, with assay of carbamazepine according to the method of Least et al (1975).

The extraction method used was based on that of Goudie and Burnett (1972), which was found to give satisfactory extraction for all these drugs and metabolites. The

conditions used for chromatography of the methylated derivatives (with addition of p-tolyl-phenytoin as internal standard) were also those used by these workers (but a flame ionisation detector was used rather than a nitrogen detector), while the trimethylsilyl derivative of PEMA was chromatographed according to the method of Baumel et al (1972). No internal standard was added for assay of PEMA, the peak height being measured in relation to the peak height of primidone, which can also be measured by this method.

Assay of HPPH

Chapter 6 describes measurement of HPPH in the urine, which is present mainly as the glucuronide conjugate rather than the free drug. GLC assay of HPPH initially requires hydrolysis of this conjugate. This can be done by acid hydrolysis - boiling in HCl but this causes problems in that the pH must be adjusted before extraction by adding saturated NaOH and this is somewhat tedious. Thus in this study enzymatic hydrolysis with β -glucuronidase was used.

Assay of Sodium Valproate and Clonazepam

The other drug which was measured by GLC in this study was sodium valproate. This is a fatty acid which is present in the serum at fairly high levels at therapeutic doses and can be readily assayed as the underivatised compound and only a simple extraction into organic solvent is necessary. The measurement of clonazepam in serum presents considerably greater difficulties, since the drug is present only in nanogram quantities, and an electron capture detector is necessary. Since this piece of apparatus was not available, clonazepam was measured in this study by radioimmunoassay.

Blood Sampling

Blood samples for measurement of serum anticonvulsants were obtained by venepuncture from an antecubital vein. Blood was collected into plain plastic tubes and the serum separated and stored at -20C until the time of assay. Half-lives in the body for phenytoin and phenobarbitone are relatively long and serum levels of these drugs vary little throughout 24 hours; blood samples for assay of these drugs in the study described in Chapter 3 were taken between 2 and 12 hours after the last drug dose. Primidone and sodium valproate have short half-lives and the blood samples taken for assay of these drugs, and also of clonazepam, were generally taken soon (2 - 4 hours) after the last drug dose.

SUMMARY

1. Methods used for the assay of anticonvulsant drugs are surveyed.
2. Gas chromatographic techniques for the assay of anticonvulsant drugs, including the particular methods used for the work described in this thesis, are described.

Note :All measurements of anticonvulsants described in this thesis were carried out by the author personally except for radioimmunoassays of phenytoin and clonazepam. The phenytoin radioimmunoassay was done by Dr J W Paxton of the Department of Materia Medica and the clonazepam radioimmunoassay was done by Dr D A O'Kelly at the Psychoendocrine Centre, St James's Hospital, Dublin.

CHAPTER 3

SURVEY OF ANTICONVULSANTS USED AND THEIR SERUM CONCENTRATIONS IN PATIENTS STUDIED FOR THIS THESIS

INTRODUCTION

The chief reason for measuring serum drug concentrations is that concentrations produced by standard doses of some drugs vary widely from patient to patient. This variation is due to differences in absorption, distribution, metabolism or excretion of the drug. For most drugs this pharmacokinetic variation is much greater than the pharmacodynamic variation (differences in tissue response to a given circulating concentration of the drug). It would be expected that the ability to monitor the serum concentration will enable the clinician to tailor the patient's dose to produce a concentration within the therapeutic range.

This Chapter describes a study which was conducted in order to examine what anticonvulsant drugs two groups of epileptic patients (inpatients and outpatients) were receiving and how the serum concentrations varied. It was found that serum concentrations of phenytoin in particular showed a high degree of variability. How does this variability arise? It was attempted to identify how various parameters (including the weight, age and sex of the patient and the administration of other drugs) altered the relationship between dose and serum concentration.

PATIENTS AND METHODS

The investigations carried out for this and succeeding Chapters were on patients receiving anticonvulsant therapy for various forms of epilepsy. They were mainly inpatients at the Epilepsy Centre, Quarriers' Homes, Bridge of Weir, Renfrewshire and outpatients attending the Institute of

Neurological Sciences, Southern General Hospital, Glasgow. A survey was carried out of what drugs these patients were receiving and blood samples for anticonvulsant analysis were obtained from most patients, on at least one occasion.

In all patients the age and sex were recorded at the time of sampling and in the inpatients and some of the outpatients the weight was also obtained. Seizure control in the inpatients was evaluated by calculating the average number of major or minor seizures per month during the period prior to blood sampling (3-12 months) in which the drug regime had remained unchanged. Serum concentrations of phenytoin, phenobarbitone and primidone were determined by GLC (see Chapter 2 and Appendix).

RESULTS

Frequency of Drug Use

The drugs which were being received by 124 inpatients and 430 outpatients are listed in Table 3.1. The outpatients were receiving a mean of 1.8 drugs and the inpatients, who were generally suffering from more severe epilepsy, were receiving a mean of 2.0 drugs. Phenytoin, phenobarbitone and primidone were by far the most commonly used anticonvulsants and Table 3.2 gives the frequencies of combinations of these drugs.

Reproducibility of Serum Concentrations

In order to determine to what extent measured serum concentrations varied on different occasions, blood samples for analysis of phenytoin, phenobarbitone or primidone were obtained on two occasions, the drug regime being unchanged. Approximately half the samples were obtained from inpatients, the other half from outpatients. Correlating the results obtained on the first and second occasions, for 86 patients on whom serum phenytoin was measured $r = 0.892$, for 59 patients in whom phenobarbitone was measured $r = 0.776$, for 37 patients in whom primidone was measured $r = 0.705$.

DRUG	INPATIENTS	OUTPATIENTS
Phenytoin	86	333
Phenobarbitone	61	202
Primidone	57	116
Diazepam	8	17
Ethosuximide	1	8
Clonazepam	4	27
Carbamazepine	21	34
Sodium Valproate	2	19
Sulthiame	4	4
Pheneturide	2	7
Total	248	767

Table 3.1 Anticonvulsant Drugs Received by 124 Inpatients and 430 Outpatients

DRUG COMBINATIONS	INPATIENTS	OUTPATIENTS
Phenytoin	13	125
Phenobarbitone	12	48
Primidone	19	30
Phenytoin + Phenobarbitone	42	136
Phenytoin + Primidone	31	68
Phenobarbitone + Primidone	5	9
Phenytoin + Primidone + Phenobarbitone	2	9
Total	124	425

Table 3.2 Combinations of Phenytoin, Phenobarbitone and Primidone received by 124 Inpatients and 430 Outpatients (5 of the Outpatients were receiving Clonazepam only)

There was no significant difference between reproducibility for outpatients and inpatients. The low correlation obtained for phenobarbitone concentrations as compared to phenytoin concentrations was due to the relatively small variation in phenobarbitone concentrations between different patients; in fact, there was a mean variation in phenobarbitone concentrations between the two occasions of only 10%. The low correlations for primidone concentrations can be explained by its short half-life and the fact that variations in the time of sampling in relation to the previous dose may produce widely different results. For further discussion of serum primidone concentrations, see Chapter 5.

Serum Phenytoin Concentrations

Serum phenytoin concentrations plotted against dose are shown in Fig 3.1 for 262 outpatients and in Fig 3.2 for 85 inpatients. There was no significant difference between the results for the two groups of patients, except that a few outpatients showed zero phenytoin concentrations on reasonably high doses of the drug and this was not found in the inpatients. There was a wide scatter of phenytoin concentrations in patients on the same dose and the distribution appeared to be log-normal; for the 83 inpatients, correlating serum concentrations against dose $r = 0.240$ ($p < 0.05$) and correlating log serum concentrations against dose $r = 0.283$ ($p < 0.01$).

The weight of the patient was obtained in 70 patients (inpatients and outpatients) who were receiving 300mg phenytoin daily. The serum concentrations were plotted against the weight and the results are shown in Fig 3.3. A significant negative relationship was found between the weight and log serum concentrations ($r = 0.368$, $p < 0.01$). The weight of the patient was also obtained for 76 patients on doses of phenytoin other than 300mg, and Fig 3.4 shows the serum concentration plotted against the weight-related dose for a total of 146 patients. As can be seen, there was no significant difference between men and women for the

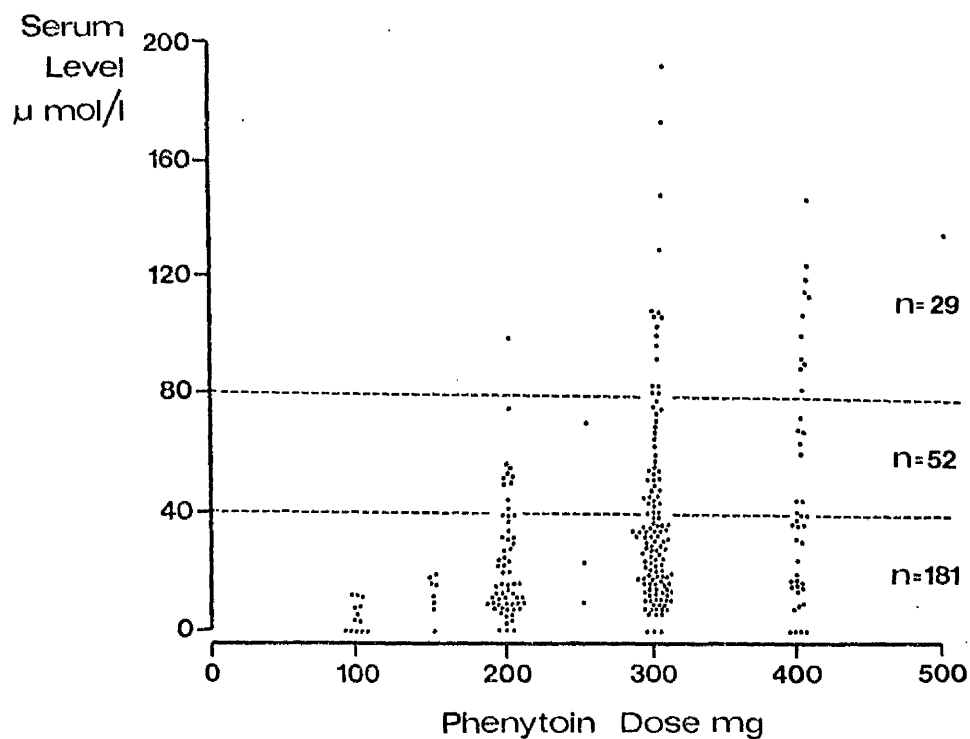


Fig 3.1 The relationship between phenytoin dose and serum concentrations in a group of 262 outpatients

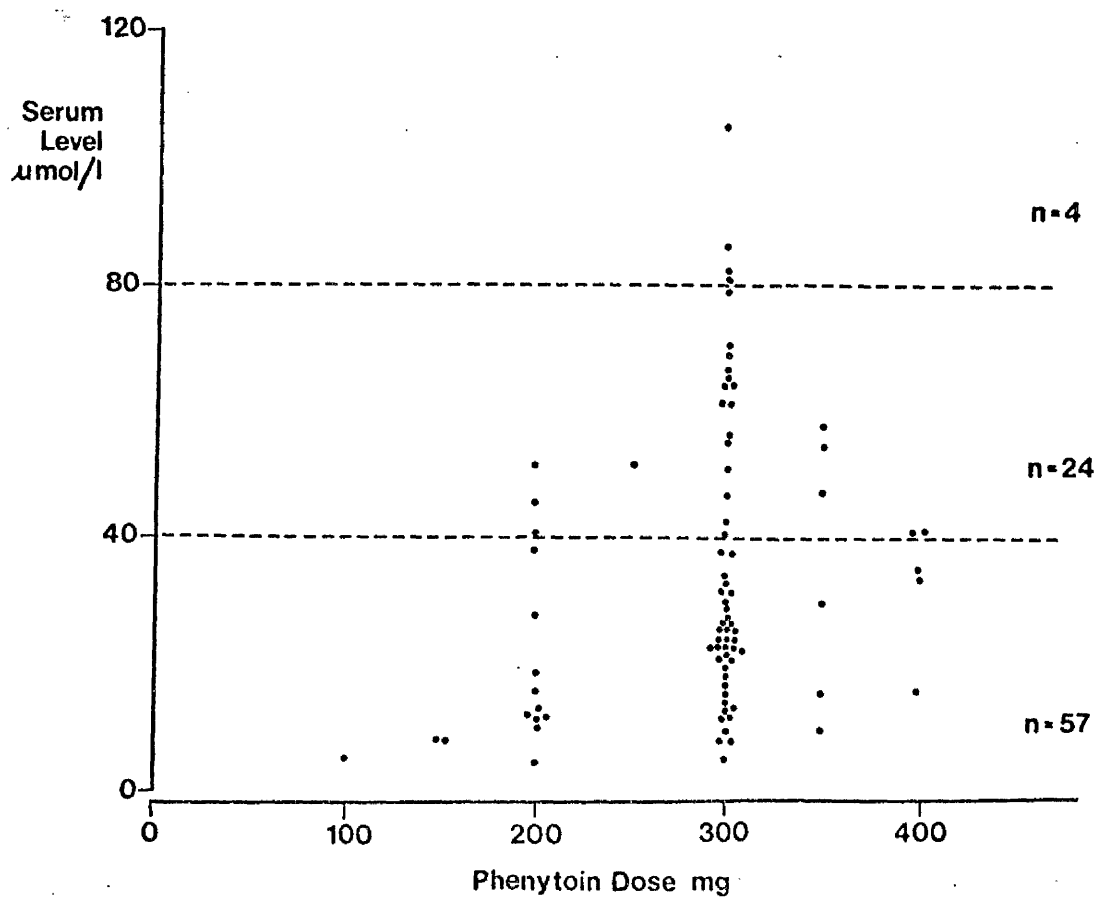


Fig 3.2 The relationship between phenytoin dose and serum concentrations in a group of 85 inpatients

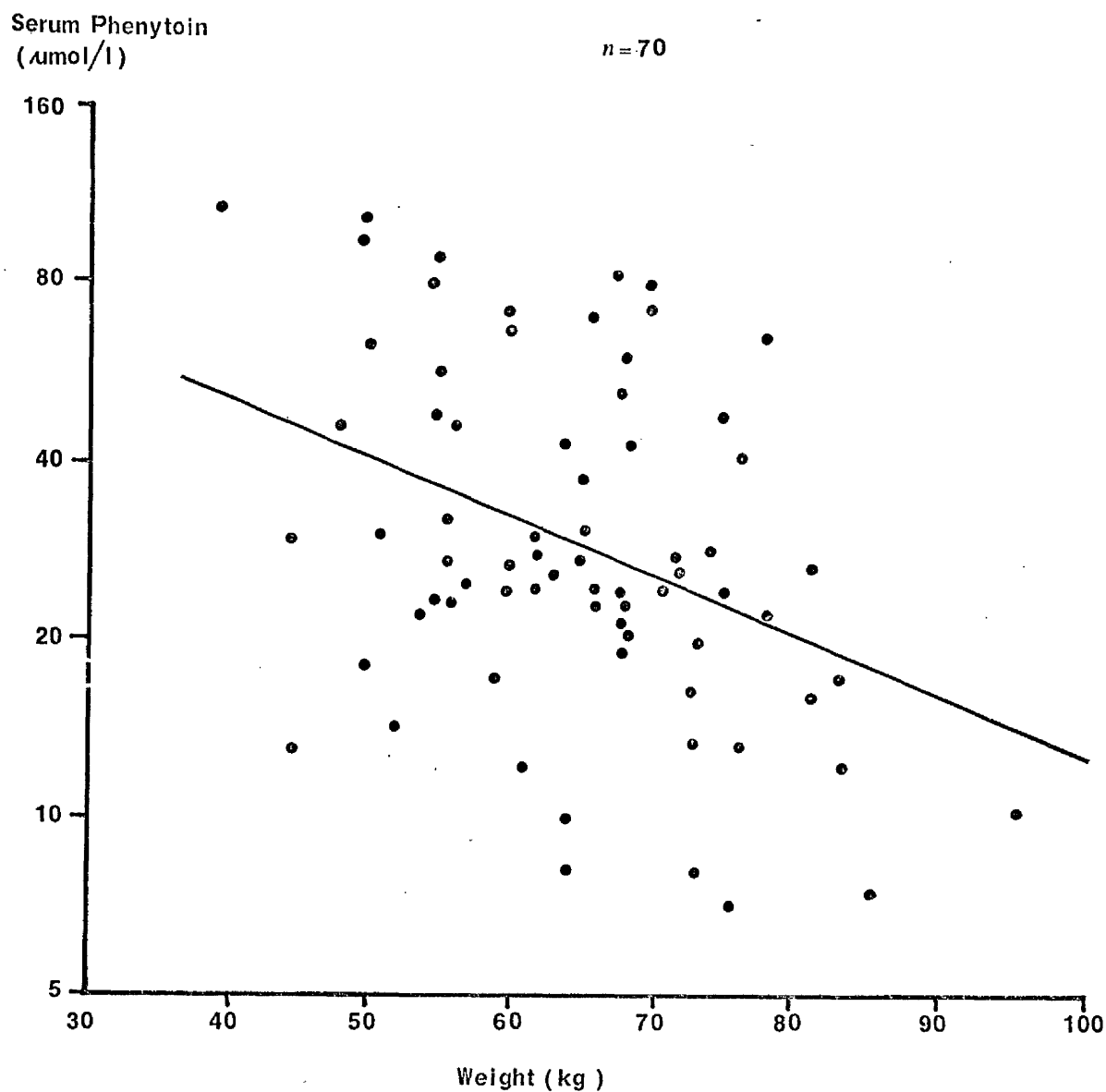


Fig 3.3

The relationship between serum phenytoin concentrations (plotted on a logarithmic scale) and the weight of the patient for 70 patients receiving 300mg of phenytoin daily. A significant negative relationship ($p < 0.01$) is shown

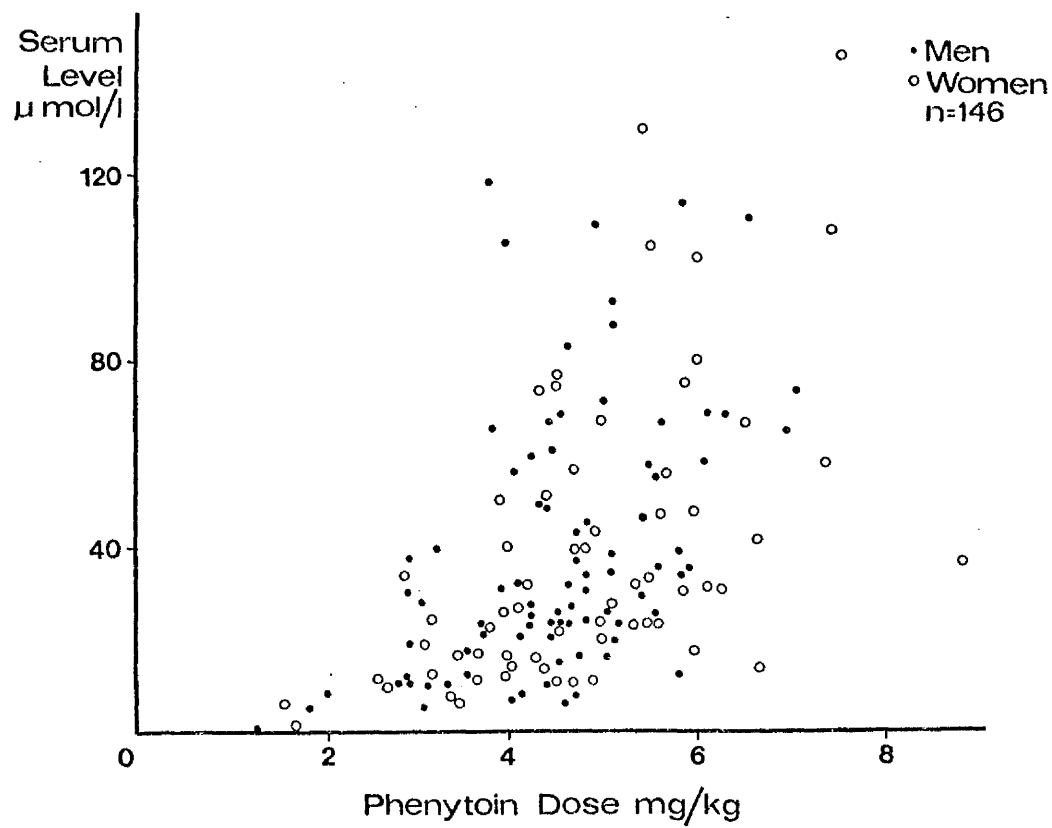


Fig 3.4 The relationship between serum phenytoin concentrations and the weight-related dose of the drug, in a group of 146 patients

relationship between phenytoin concentration and weight related dose. Fig 3.5 shows the serum phenytoin concentration plotted on a logarithmic scale against age for patients receiving 300mg phenytoin. A small but significant positive relationship was found ($r = 0.251$, $p < 0.01$).

It is also possible that the drug interactions may cause an increase or decrease in serum phenytoin concentrations and may increase variability. A higher correlation between phenytoin dose and log serum concentration was obtained for 68 patients on single drug therapy with phenytoin ($r = 0.389$, $p < 0.01$) compared to the 83 inpatients described above, who were mainly on combination therapy with phenobarbitone or primidone. Serum concentrations of phenytoin in patients on single therapy and combined therapy with phenobarbitone are shown in Fig 3.6. There is slightly more variation in serum concentrations in patients on combined therapy. There is no difference in the mean serum concentrations between the two groups of patients, comparing patients receiving 300mg phenytoin daily; 27 patients on single therapy showed a geometric mean phenytoin serum concentration of $35.8 \mu\text{mol/l}$ and 46 patients receiving phenobarbitone showed a geometric mean phenytoin serum concentration of $33.9 \mu\text{mol/l}$. 36 patients receiving 300mg phenytoin and receiving primidone, however, showed a lower geometric mean phenytoin serum concentration of $26.9 \mu\text{mol/l}$; comparing phenytoin serum concentrations in patients receiving phenytoin alone and patients receiving primidone, the difference between the means did not quite reach significance ($t = -1.616$, $p < 0.1$, comparing the log values of the serum concentrations).

Phenobarbitone serum concentrations were plotted against phenytoin serum concentrations for the 58 patients on combined therapy and on a standard dose of 300mg phenytoin daily. There was no significant correlation between the serum phenobarbitone concentration and the log serum concentration of phenytoin. However, there is evidence that, at least in patients on phenytoin, the phenobarbitone serum concentrations show an approximately log - normal distribution (see following section). Correlating the logarithms of the serum concentrations of phenytoin and phenobarbitone, a

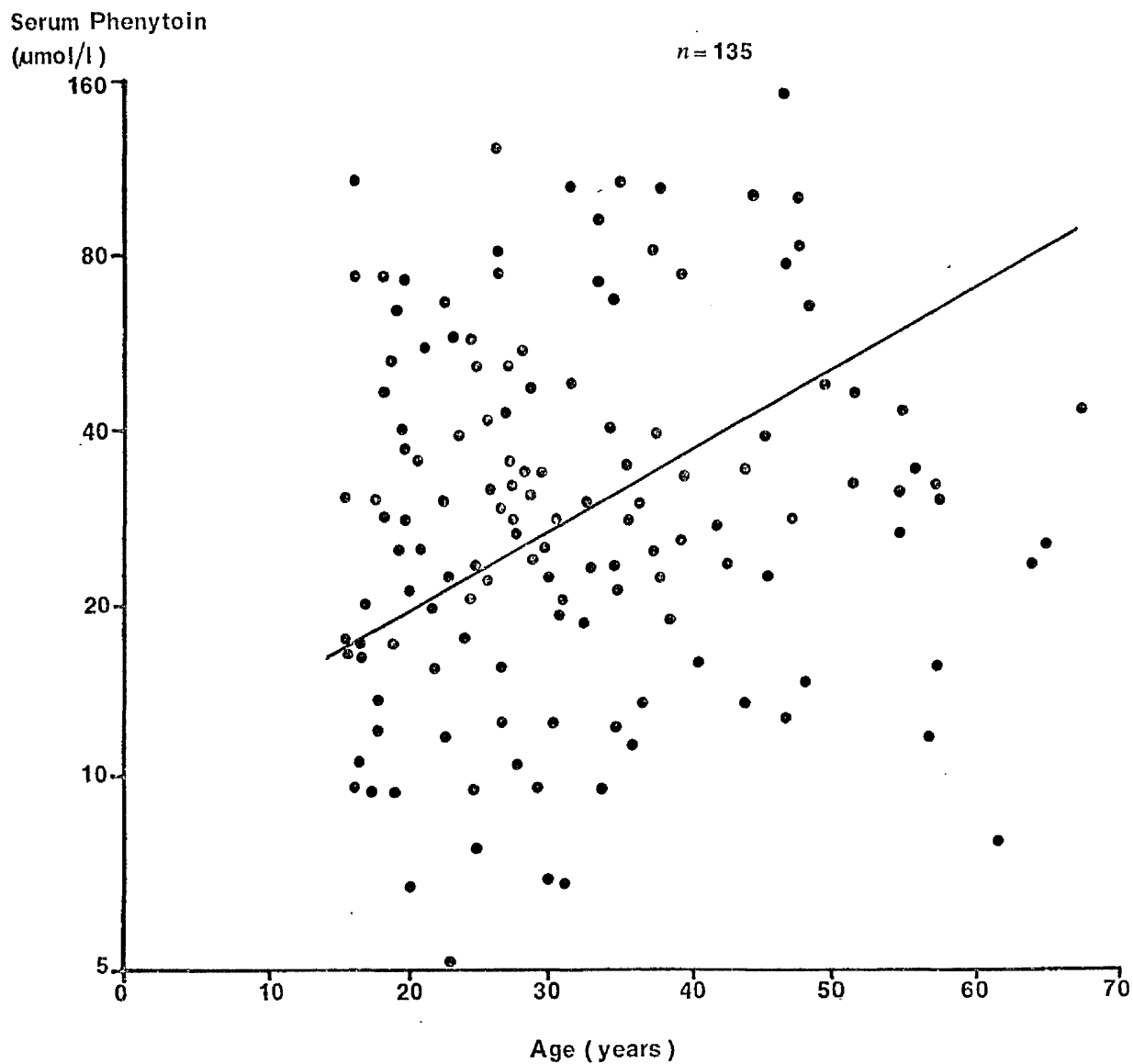


Fig 3.5 The relationship between serum phenytoin concentrations plotted on a logarithmic scale and the age of the patient for 135 patients receiving 300mg phenytoin daily. A significant positive relationship ($p < 0.01$) is shown

significant positive relationship was found ($r = 0.358$, $p < 0.01$).

Serum Phenobarbitone Concentrations

Phenobarbitone showed less variability in serum concentrations than phenytoin with the weight-related dose.

For 49 patients in whom the weight was obtained, correlating serum concentrations with dose $r = 0.793$

There was a particularly high correlation between dose and serum concentration for patients receiving phenobarbitone alone. This can be seen in Fig 3.7, where results for 22 patients receiving single therapy and 78 patients receiving combined therapy are plotted; none of these patients were receiving any other drugs. The equation of the line for patients receiving phenobarbitone alone was

serum concentration = $12.6 + 0.46 \times \text{dose}$ ($r = 0.813$)

and for patients receiving phenytoin was

serum concentration = $2.5 + 0.78 \times \text{dose}$ ($r = 0.663$)

Patients receiving phenytoin had significantly higher phenobarbitone serum concentrations and the lower r value shows that there was greater variability. In fact, in patients on phenytoin there was evidence that the serum concentrations did not show a normal distribution but rather approximated to a log-normal distribution.

Seizure Control

Seizure frequency was assessed in 57 patients who were on a standard dose of 300mg phenytoin daily. All but two were receiving phenobarbitone or primidone also and a number were receiving other drugs - carbamazepine (7), diazepam (3), clonazepam (1) and sulthiame (1). The seizure frequency in relation to serum concentration of phenytoin is shown in Table 3.3. There was no significant relationship between serum concentration and seizure frequency but patients showing concentrations below $40 \mu\text{mol/l}$ were having more major seizures.

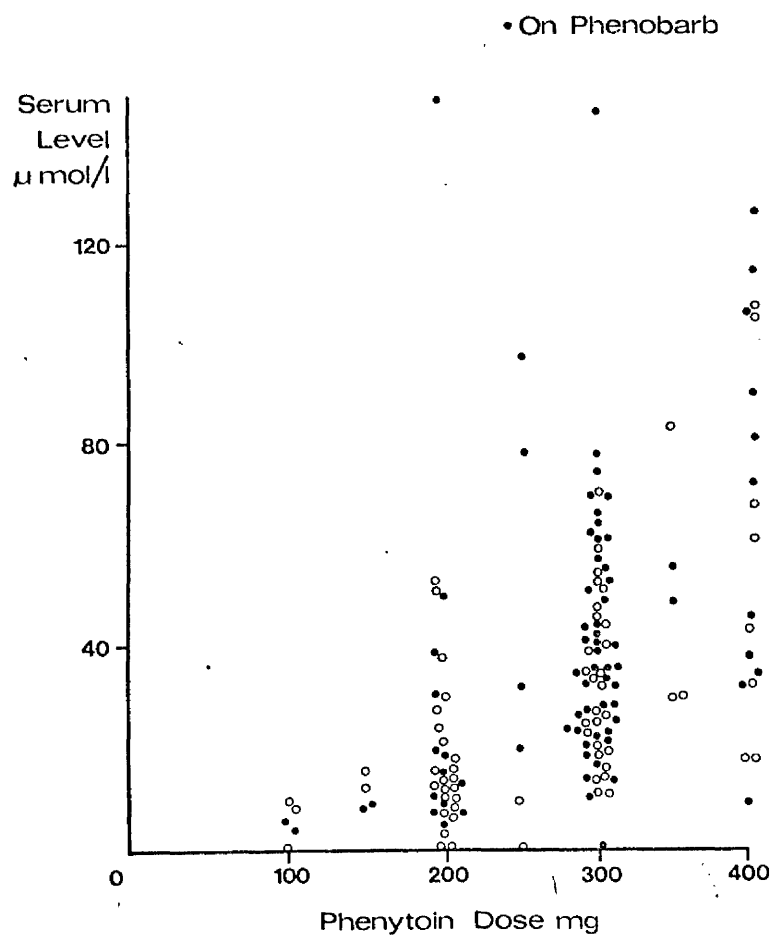


Fig 3.6

The relationship between phenytoin dose and serum concentration, for patients receiving the drug alone and patients receiving combined therapy with phenobarbitone

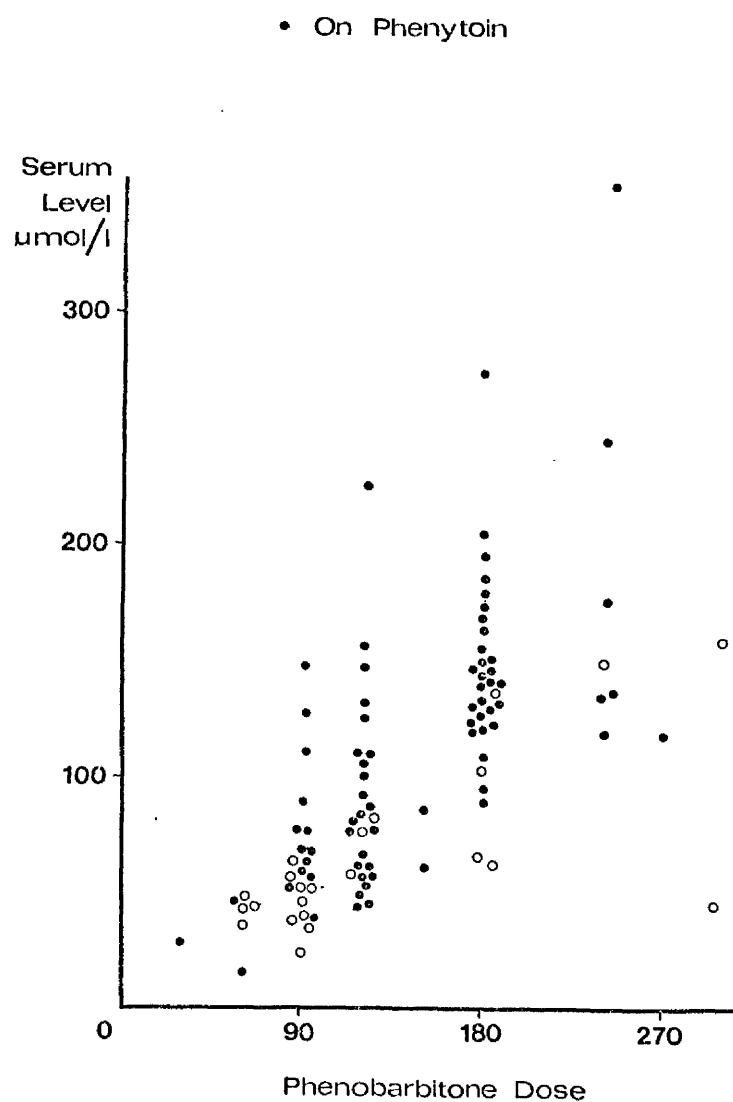


Fig 3.7

The relationship between phenobarbitone dose and serum concentration, for 22 patients receiving the drug alone and 78 patients on combined therapy with phenytoin

Phenytoin Concentration Range	No of Patients	Major Seizures	Minor Seizures
0 - 40 $\mu\text{mol/l}$	38	1.82	8.0
40 - 80	16	1.04	10.1
> 80	3	0.3	2.2

Table 3.3 Mean Seizure Frequency Per Month Per Patient for Groups of Patients Showing Phenytoin Serum Concentrations Below, In and Above the Therapeutic Range

DISCUSSION

By far the most commonly used drugs in the patients described in this study were phenytoin, phenobarbitone and primidone. Combinations of phenytoin with phenobarbitone or primidone were particularly common. Since phenytoin, phenobarbitone and primidone were by far the most commonly used drugs, these drugs were selected for studies of their serum concentrations which are described in this and the succeeding two Chapters. Although clonazepam and sodium valproate were infrequently used in these patients, studies of these drugs were conducted and are described in Chapters 6 and 7 because they had only recently been introduced at the time this survey was carried out and it is likely that they will be of increasing importance. Note that the patients in this study were all over 16 years of age; it is likely that a younger population would be receiving ethosuximide more frequently, for treatment of petit malepilepsy.

The combination drug therapy in these patients is notable. Similar findings were obtained in a recent survey of 11,720 patients in 15 centres in 4 European countries (Guelen et al, 1975), it being found that the patients were receiving a mean of 2.7 anticonvulsant drugs Shorvon and Reynolds (1977) recently drawn attention to this tendency towards poly-

pharmacy as an unsatisfactory aspect of the drug treatment of epilepsy. There is a lack of evidence that, for instance, the widely practiced treatment of epilepsy with phenytoin and phenobarbitone offers advantages over single drug therapy. This and succeeding Chapters present evidence that the apparent potentiation when phenytoin and phenobarbitone (Chen and Ensor, 1954; Weaver et al, 1955) and possibly also when phenytoin and primidone are used in combination may be due to drug interactions which increase serum concentrations of one or both drugs. In Chapter 6 it is shown that administration of other drugs may reduce the value of clonazepam therapy and in Chapter 7 it is shown that a drug interaction between sodium valproate and phenobarbitone may cause toxic effects.

Serum Phenytoin Concentrations

Serum concentrations of phenytoin showed particularly wide variation between different patients. This is in line with a large number of other studies (eg Lascalettes et al, 1970; Kutt, 1971; Buchthal et al, 1972). It appears that the enzyme responsible for phenytoin metabolism becomes saturated at higher serum concentrations (Bochner et al, 1972) and this exaggerates the variation in the rate of metabolism in different patients which is produced by genetic influences. As the dose of phenytoin is increased, the increments in serum concentration become larger.

The distribution of serum phenytoin concentrations was log-normal, as found by other workers (Houghton et al, 1975), with most patients showing serum concentrations below the proposed therapeutic range of 40 - 80 $\mu\text{mol/l}$ (Buchthal et al, 1960). It is unlikely that the low concentrations in most instances were due to failure of compliance since inpatients, in whom drug intake was closely supervised, showed similar concentrations; however, a few outpatients showed zero concentrations on fairly high doses which may have been due to failure to take the drug. A retrospective study was carried out to determine whether patients showing serum phenytoin concentrations below the therapeutic range showed poorer control - a trend towards a higher frequency

of major but not minor seizures was found in patients with low concentrations below 40 $\mu\text{mol/l}$. A study was carried out and is described in the following Chapter which shows that increasing serum concentrations into the therapeutic range improves control of major seizures.

Thus the variation in serum concentrations of phenytoin may be of therapeutic importance. It is of course possible to adjust serum concentrations into the therapeutic range by routine assay, but it would be desirable to predict more accurately the serum concentration that would be obtained on a given phenytoin dose. It might be expected that this would be made easier if one could allow for the effect of various parameters which alter the relationship between phenytoin dose and serum concentration. Such parameters include the weight and age of the patient and the effects of administration of other drugs.

Serum phenytoin concentrations measured in patients receiving 300mg of the drug daily showed a negative correlation with body weight, similar to that found by Houghton et al (1975). However, the low value of the correlation coefficient indicates that this accounts for only a small part of the variation in serum phenytoin concentrations between patients. Friedman et al (1960) found similar results and concluded that an accurate estimate of the serum phenytoin concentration could not be made from the weight-related dose.

A positive correlation between age and serum concentrations of phenytoin was found in patients on a standard dose of 300 mg daily. A similar finding was reported by Houghton et al (1975) and in another study it was shown that a group of young patients showed considerably lower phenytoin concentrations than a group of older patients (Haerer and Grace, 1969). Other workers have also found that children require a larger dose of phenytoin/kg of body weight than adults in order to obtain therapeutic concentrations (Jalling et al, 1970; Dawson and Jamieson, 1971). The most likely explanation for the finding of increased phenytoin in the aged is a reduction in the rate of metabolism. This is examined in Chapter 4A.

Patients receiving phenobarbitone in this study showed similar serum phenytoin concentrations to those receiving phenytoin alone, suggesting that there is a significant overall effect of phenobarbitone on phenytoin metabolism. Interaction between phenobarbitone and phenytoin has been studied by a number of workers with varying results (Table 3.4). Some patients show an increase and some a decrease in phenytoin serum concentrations when phenobarbitone is added. It is likely that two opposing effects occur - competitive inhibition of phenytoin metabolism occurs with the start of phenytoin therapy, but a more slowly developing induction of the liver metabolising enzymes reverses this effect. Phenobarbitone serum concentrations were correlated with phenytoin serum concentrations in 58 patients on combined therapy and on a standard dose of 300mg phenytoin daily. Correlating the logarithms of the serum concentrations of both phenytoin and phenobarbitone, a significant positive relationship was found. This would suggest that serum concentrations are increased by inhibition of metabolism in patients with high concentrations of phenobarbitone. However, it is likely that the opposite effect also occurs and that serum phenobarbitone concentrations are increased in patients with high serum phenytoin concentrations - see the following section and Chapter 4.

Although there is no overall effect of phenobarbitone on serum concentrations of phenytoin, a possible effect of primidone was found. Patients receiving primidone showed generally lower serum phenytoin concentrations than patients receiving phenytoin alone and this almost reached significance. It is possible that in patients receiving primidone there is a greater induction effect on phenytoin metabolism relative to the inhibition produced by the derived phenobarbitone than for patients receiving phenobarbitone. Evidence that this is indeed likely is presented in Chapter 11, where it is shown that patients receiving primidone have a higher level of enzyme induction than patients with comparable serum concentrations of phenobarbitone who are receiving phenobarbitone itself.

In conclusion, the weight and age of the patient and the

REFERENCE	NO OF PATIENTS	PHENYTOIN LEVEL BEFORE PHENOBARBITONE ADMINISTRATION		PHENYTOIN LEVEL AFTER PHENOBARBITONE ADMINISTRATION		PHENYTOIN CONCENTRATION CHANGE
		Range	Mean	Range	Mean	
Cucinell et al (1965)	5	28 - 88	44	16 - 20	28	Decrease
Buchanan et al (1969)	4	12 - 48	26.4	4 - 28	10.4	Decrease
Hirschmann (1969)	1	-	28	-	28	None
Kutt et al (1969)	1	-	68	-	112	Increase
	9	20 - 92	52	- 68	40	Decrease
	11	12 - 76	36	- 76	36	None
	6	16 - 72	36	- 88	44	Increase
Diamond and Buchanan (1970)	4	6 - 24.4	17.6	2.8 - 18.8	11.6	Decrease
	3	3.6 - 14	13.2	3.6 - 14	13.2	None
	3	2.4 - 30	16.4	4.4 - 32.4	20	Increase
Garrettson and Dayton (1970)	2	13.2 - 21	48	1.6 - 48	24	Decrease
	1	-	11.2	-	11.2	None
	1	-	11.2	-	15.6	Increase
Morselli et al (1971)	5	16 - 160	64	4 - 68	24	Decrease
	2	20 - 32	26	- 32	26	None
Booker et al (1971)	8	Unknown	60.4	Unknown	78	Increase

Table 3.4

Reported Effects of Phenobarbitone on Phenytoin Serum Concentrations ($\mu\text{mol/l}$) in Human Patients. Modified from Kutt and Louis (1972)

administration of other drugs may all affect the relationship between serum concentration and dose of phenytoin. However, even allowing for these factors there is a great deal of variability in serum concentrations of phenytoin in different patients on the same dose. It is possible that serum concentrations may be more predictable in a given patient at a range of doses if the serum concentration is measured at one dose - this is examined in the following Chapter.

Serum Phenobarbitone Concentrations

By contrast with phenytoin, phenobarbitone serum concentrations showed a high correlation with dose, particularly with the weight-related dose.

Patients receiving phenytoin in this study showed significantly higher concentrations of phenobarbitone than patients receiving phenobarbitone alone. A similar finding has been reported by other workers (Morselli et al, 1971) and it is likely that phenytoin acts as a competitive inhibitor of phenobarbitone metabolism. Further evidence for this interaction is given in the following Chapter, it being found that increase in phenytoin dose and serum concentrations also caused increased serum phenobarbitone concentrations. The interactions between phenytoin and primidone, which is partly metabolised to phenobarbitone, are complex and these are discussed separately in Chapter 5.

SUMMARY

1. A survey was made of the anticonvulsant drugs that two groups of epileptic patients (inpatients and outpatients) were receiving and of the serum concentrations of the drugs. The seizure control in the inpatients was also assessed.
2. Serum concentrations of phenytoin showed particularly wide variation between different patients. There was no significant relationship between serum concentrations and seizure control,

although patients showing concentrations below the accepted therapeutic range (40 - 80 $\mu\text{mol/l}$) had more major seizures.

3. The effects of various parameters on serum concentrations of phenytoin in patients on a fixed dose (300mg) of the drug were studied. A significant negative correlation was found between serum concentration and the weight of the patient and a positive correlation was found with age. There was no difference in serum concentrations between patients receiving phenobarbitone and patients receiving phenytoin alone, although patients receiving primidone showed lower concentrations.
4. Serum phenobarbitone concentrations were significantly higher in patients receiving phenytoin than in patients receiving phenobarbitone alone. There was a significant positive correlation between phenobarbitone and phenytoin serum concentrations in patients receiving both drugs, probably due to both an inhibition by phenytoin of phenobarbitone metabolism and inhibition by phenobarbitone of phenytoin metabolism.

CHAPTER 4

HOW DO SEIZURE CONTROL AND SERUM CONCENTRATIONS ALTER
AS PHENYTOIN DOSE IS INCREASED?

"You may object that it is not a trial at all,
you are quite right, for it is only a trial
if I recognise it as such."

from "The Trial" by Franz Kafka

INTRODUCTION

Two prospective studies of phenytoin in the treatment of epilepsy have suggested that maximum seizure control is obtained only when its concentration in serum is greater than 40 $\mu\text{mol/l}$ (Buchthal et al, 1960; Lund, 1974) and the therapeutic range is commonly regarded as 40 - 80 $\mu\text{mol/l}$ (10 - 20 $\mu\text{mol/l}$) (Lancet, 1975). However, as has been shown in Chapter 3, wide variation of serum concentrations of phenytoin is found in different individuals on the same dose and in most instances serum concentrations lie outside the therapeutic range unless adequate monitoring is performed.

Difficulties in achieving therapeutic serum concentrations of phenytoin can be explained by its pharmacokinetics. There is a non-linear relationship between dose and serum concentration of phenytoin so that serum concentration may rise rapidly with increasing dose. The relationship can be described by the Michaelis-Menten equation and defined in terms of the serum concentration at which metabolism is 50% saturated (Michaelis constant, K_m) and the maximum rate of phenytoin metabolism (V_{max}) (Fig 4.1). A nomogram has been produced which can be used to predict the therapeutic dose of phenytoin (Richens and Dunlop, 1975) (Fig 4.2). The nomogram depends upon the assumption that K_m remains relatively constant in different individuals, whereas

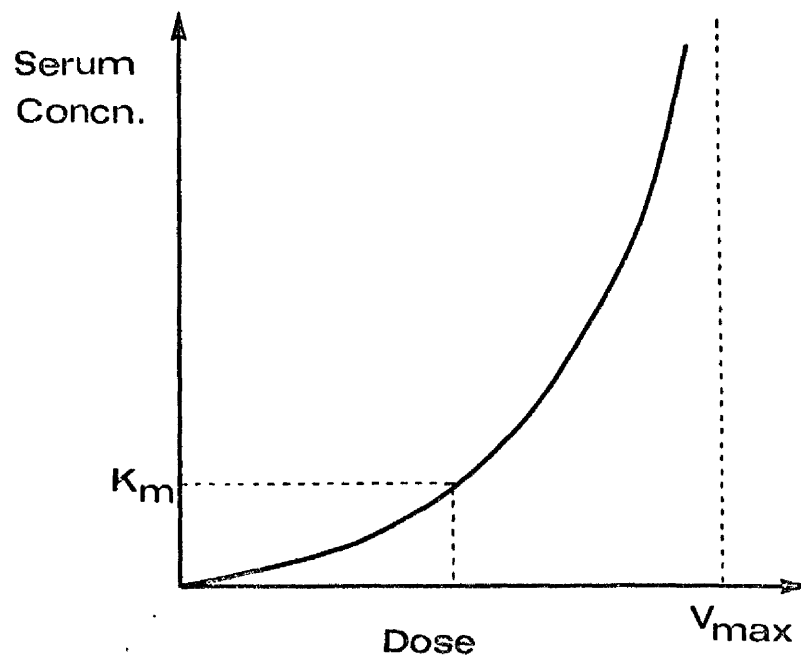
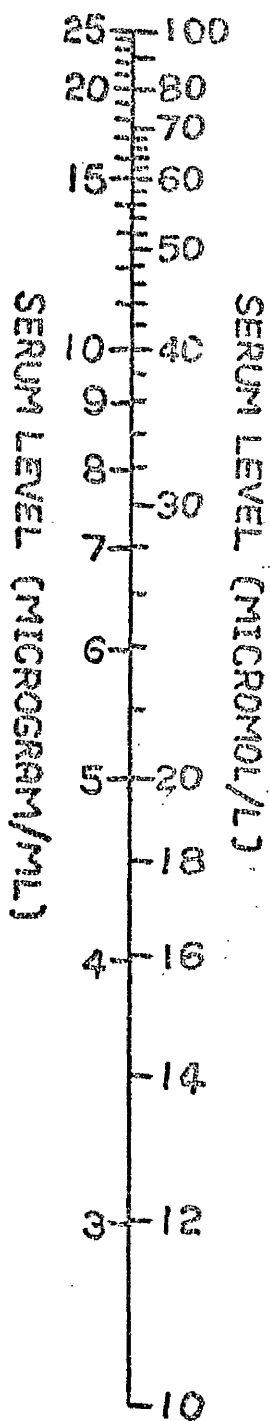
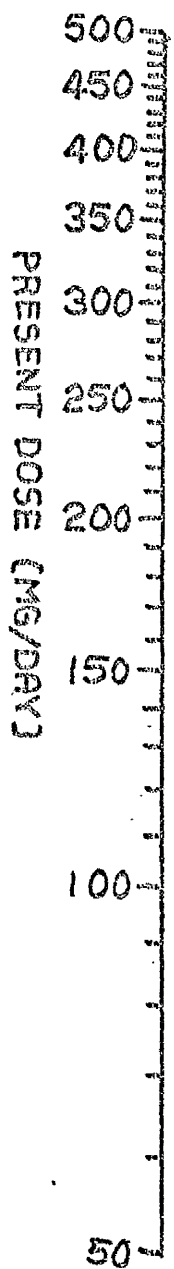
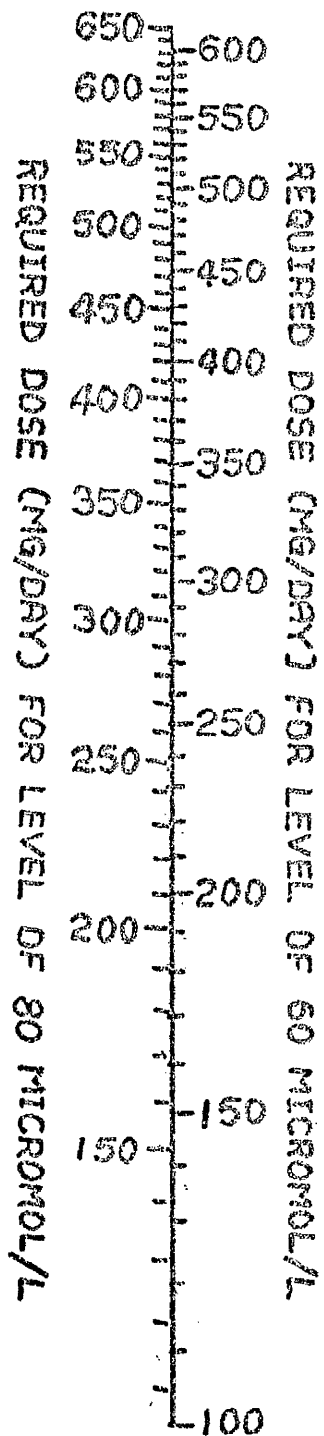


Fig 4.1 Kinetics of phenytoin described by the Michaelis-Menten relationship. V_{max} is the maximum velocity of the reaction (ie the maximum dose which can be metabolised) and K_m is the serum concentration at $\frac{1}{2} V_{max}$

Fig 4.2

Nomogram for adjusting phenytoin dosage, described by Richens and Dunlop (1975). Given a single serum concentration on a given daily dose of the drug, the dose required to achieve a serum concentration of $60\mu\text{mol/l}$ or $80\mu\text{mol/l}$ can be predicted. A line is drawn connecting the measured serum concentration (left hand scale) with the dose administered (centre scale), and then by extending this line to the right hand scale the dose required to produce a therapeutic concentration of the drug can be read off



Vmax values vary considerably because of variation in the degree of enzyme induction.

The therapeutic range of phenytoin serum levels has been established mainly in patients receiving phenytoin alone, but many patients receive phenytoin in combination with other anticonvulsants, particularly phenobarbitone or primidone. Also, previous studies have supported the relationship between serum levels and control of grand mal seizures (Buchthal et al, 1960; Lund, 1973; Lund 1974); there has been no assessment of the value of adjusting serum levels in the control of absences or focal seizures. In this Chapter the relationship between phenytoin level and therapeutic effect against different types of seizure, in patients on multiple drug therapy, is examined. The value of the nomogram for predicting therapeutic dose has been assessed and the assumptions on which it is based have been studied. Also, in Chapter 3, the possible effects of administration of phenobarbitone or primidone on serum concentrations of phenytoin were discussed; in this Chapter the effect of administration of these drugs on the relationship between phenytoin dose and serum concentration in individual patients has been investigated.

PATIENTS AND METHODS

Patients

Twenty epileptic inpatients with serum levels of phenytoin below 40 $\mu\text{mol/l}$ were selected for study. There were seventeen males and three females aged 19 - 63 years. Nine patients had generalised tonic-clonic epilepsy, nine had temporal-lobe epilepsy and two had myoclonic-atonic epilepsy. All patients were having an average of at least one minor seizure per month and sixteen patients were having one or more major seizures per month. All patients were receiving phenytoin (200 - 350mg daily) and either phenobarbitone (90 - 180mg daily) or primidone (750 - 1000mg) daily.

Methods

Fig 4.3 shows the design of the study, which lasted eight months. During the first four months the drug regimen was unchanged and control observations were made of fit frequencies. All seizures were recorded throughout the trial by the nursing staff as major (tonic-clonic) or minor seizures. Serum concentrations of phenytoin, and also of phenobarbitone and primidone, were measured at the beginning and end of the control period. In the second four months the phenytoin doses were increased and blood samples for anticonvulsant analysis were obtained at monthly intervals. In fifteen patients a therapeutic serum concentration of phenytoin was obtained after one dose increase of 50 - 100mg and in five patients further dose increases were required. The doses of drugs other than phenytoin remained unaltered throughout the study.

RESULTS

Seizure Frequency

The effects of increased phenytoin therapy on the frequencies of major and minor seizures in twenty patients on multiple drug therapy are shown in Figs 4.4a and 4.4b. In patients in whom a second dose increase was necessary, seizure frequency was assessed over the last two months only. Minor seizures were unchanged but there was a reduction in major seizures in the sixteen patients affected. Seven patients with major seizures had no further seizures when the serum concentrations of phenytoin were increased above 40 $\mu\text{mol/l}$. Three other patients showed a reduction in frequency of major seizures. In three patients major seizures increased and three patients showed no change. The overall changes in seizures are summarised in Table 4.1. Toxic effects of phenytoin therapy were observed in one patient, who had the highest serum concentration of 104 $\mu\text{mol/l}$.

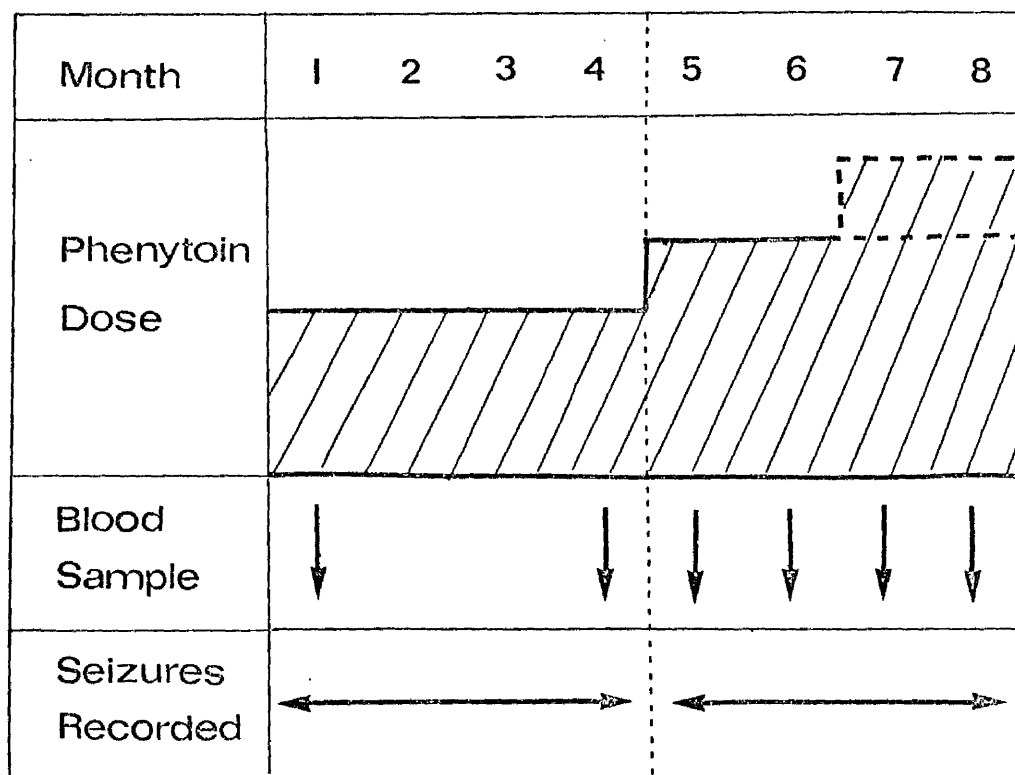


Fig 4.3 Design of the trial to investigate how altering dose of phenytoin affected serum drug concentrations and seizure control

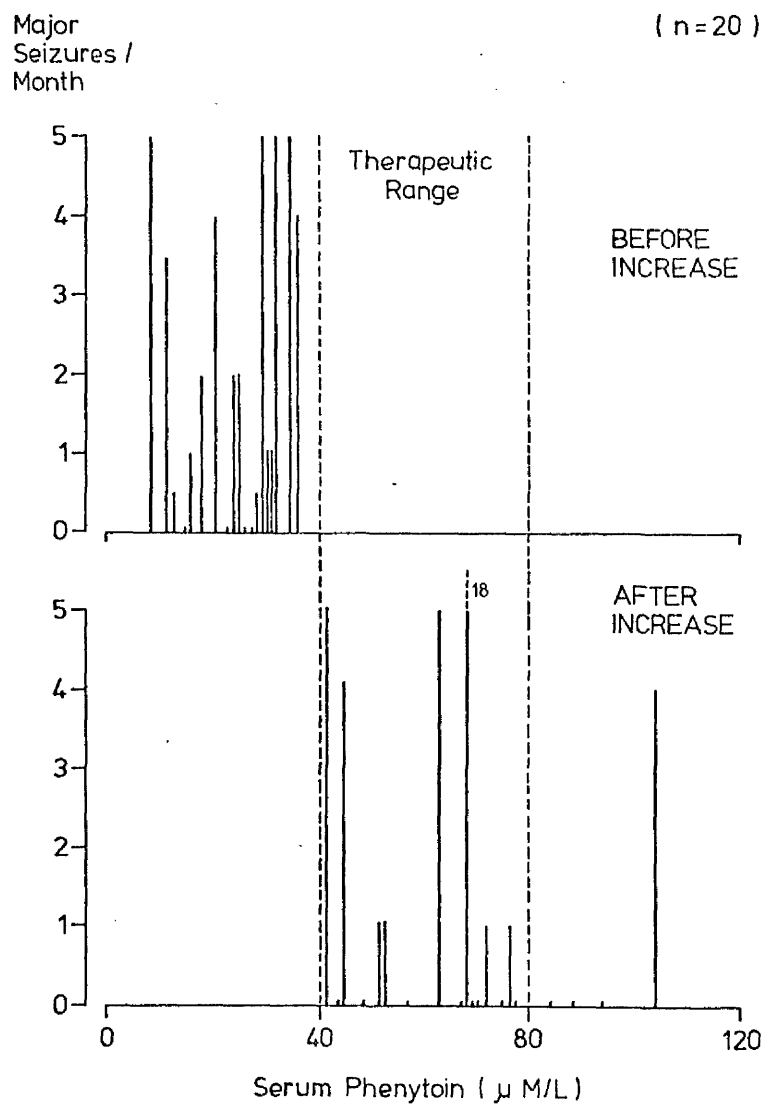


Fig 4.4a

Effect of increasing serum concentrations of phenytoin into the therapeutic range on frequency of major seizures in a group of 20 inpatients. Each vertical line represents one patient

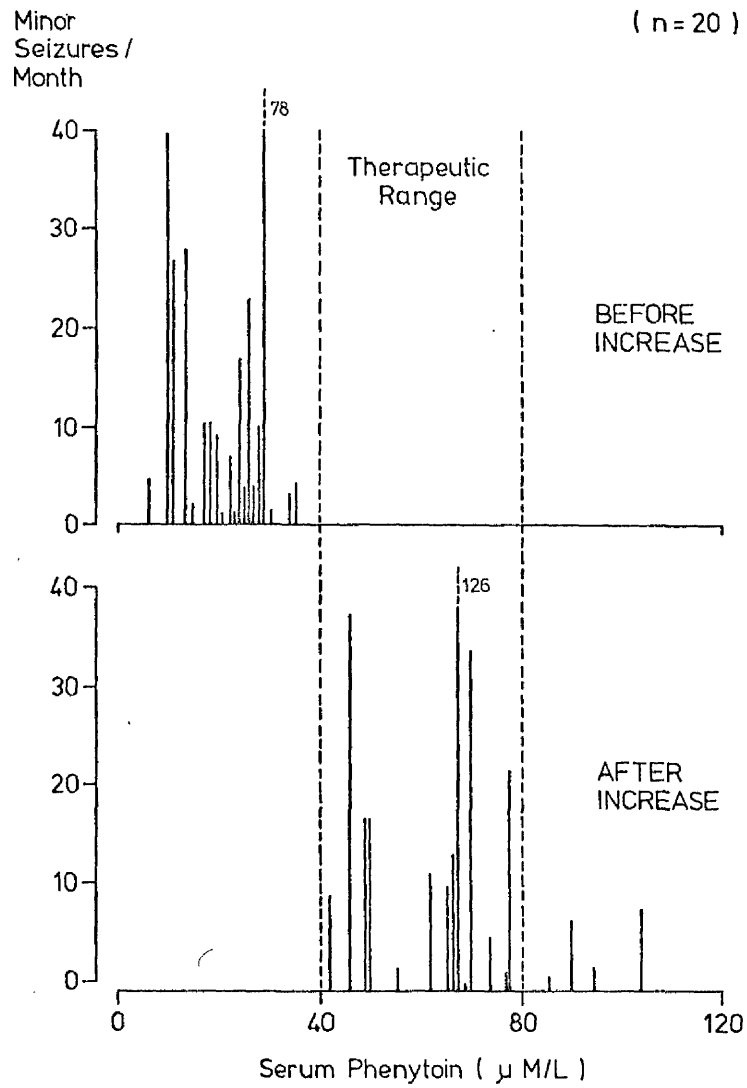


Fig 4.4b

Effect of increasing serum concentrations of phenytoin into the therapeutic range on frequency of minor seizures in a group of 20 inpatients

Type of Epilepsy	Major Seizures		Minor Seizures	
	improved	no change / worse	improved	no change / worse
Generalised	6	-	6	1
Temporal Lobe	2	3	2	1
Myoclonic-atonic	1	-	1	-
				2
				6
				1

Table 4.1
Effect of increased phenytoin therapy on control
of major and minor seizures in twenty patients

Serum Anticonvulsant Concentrations

The patients in the trial initially received total daily doses of 200 - 350mg of phenytoin and doses of 250 - 450mg daily were required to attain serum concentrations above 40 $\mu\text{mol/l}$. In a number of patients the dose required to attain therapeutic serum concentrations was considerably less than that predicted from the nomogram (Richens and Dunlop, 1975). For the eight patients who showed initial serum concentrations of phenytoin below 20 $\mu\text{mol/l}$, the predicted doses (of up to 650mg) were considerably greater than required. An average K_m in twenty patients of 2.2mg/l (range 1.4 - 4.8 mg/l) was found. The K_m values for the five patients in the study who required two dose increases are shown by the slopes of the continuous lines plotted in Fig 4.5. The slope of the interrupted line in this Figure indicates the K_m on which the nomogram was based (K_m 3.1), which is somewhat higher than the mean observation in this study. The V_{max} values in these five patients are given by the intercepts on the y-axis. A mean V_{max} in twenty patients of 375mg/day (range 250 - 550mg/day) was found; the V_{max} values were generally less variable than the K_m values, with fifteen of the twenty patients showing V_{max} values in the range 350 - 450mg/day.

Not only phenytoin concentrations but also phenobarbitone concentrations in serum increased as the phenytoin dose was increased in these patients. Serum phenobarbitone levels, both in patients receiving phenobarbitone and patients receiving primidone, significantly increased from a mean of 103 $\mu\text{mol/l}$ to a mean of 140 $\mu\text{mol/l}$ ($P < 0.01$, Wilcoxon signed-ranks test) as phenytoin dose was raised. This effect was most marked in patients in whom particularly high serum phenytoin levels were obtained and is shown graphically in Fig 4.6. All twenty patients in this trial were receiving either phenobarbitone or primidone in addition to phenytoin and, as this might affect the relationship between dose and serum level of phenytoin, a comparison was made with five patients

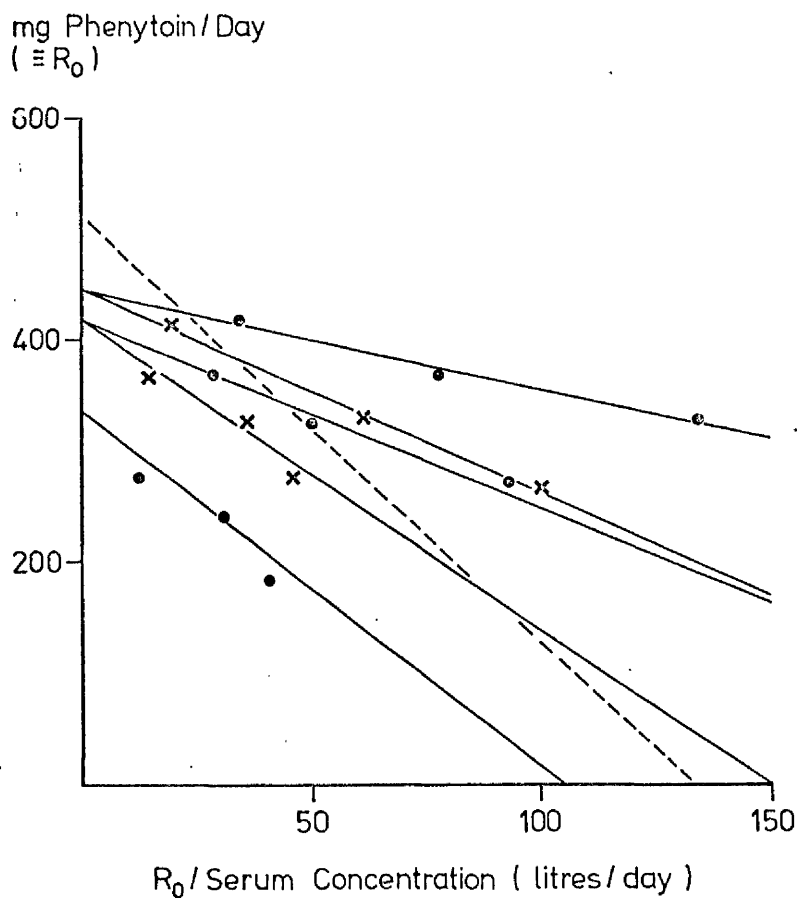


Fig 4.5

Graphic estimations of individual V_{max} and K_m values for phenytoin metabolism in five patients in whom serum phenytoin concentrations were measured at three doses. The y-intercept is the value of V_{max} and the slope is the negative value of K_m

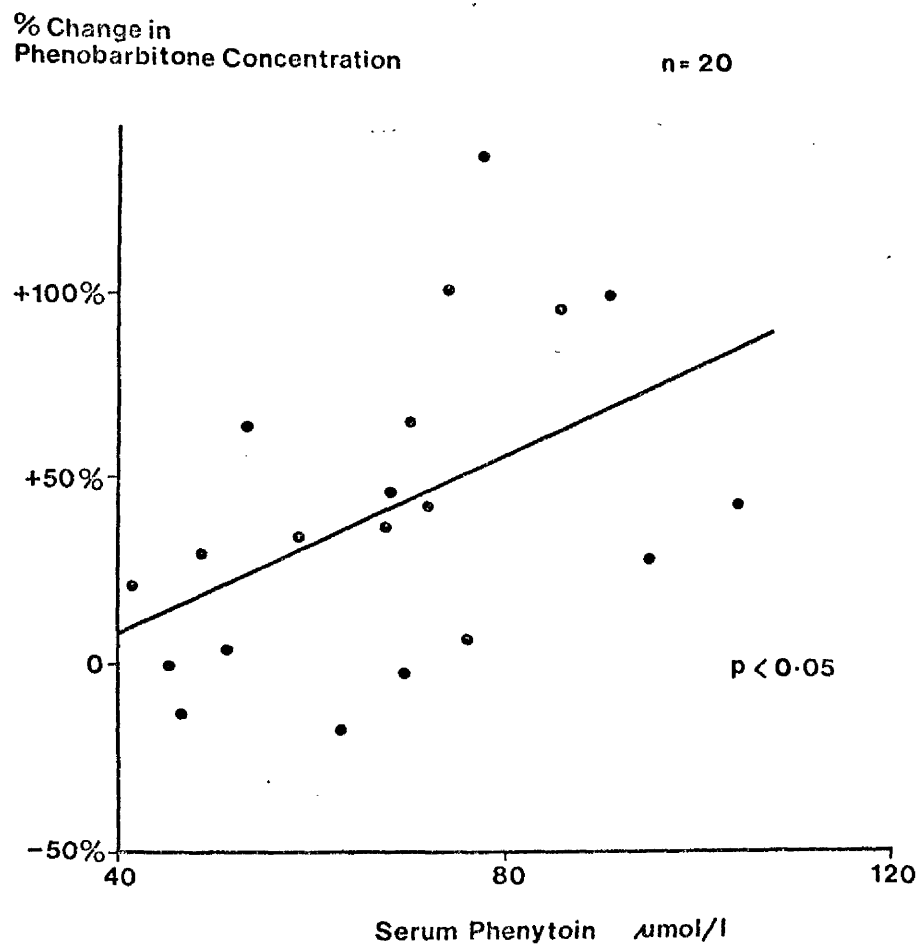


Fig 4.6 Percentage change in phenobarbitone concentrations, plotted against final serum phenytoin concentration, for 20 patients in whom serum phenytoin concentration was increased from below $40\mu\text{mol/l}$

receiving phenytoin alone. Five outpatients were studied, phenytoin doses being increased from 200 - 400mg daily to 300 - 500mg daily and the serum level of phenytoin measured at both doses. The mean K_m in these patients was 4.4 (range 2.0 - 5.6) and the mean V_{max} was 410 (range 340 - 520).

DISCUSSION

The results presented here are in line with a therapeutic range for serum phenytoin concentrations of 40 - 80 $\mu\text{mol/l}$. It was found that increase of serum concentrations above 40 $\mu\text{mol/l}$ in patients on combined therapy with phenobarbitone or primidone, improved control of major seizures although minor seizures were unaffected. That major but not minor seizures are reduced by increased phenytoin therapy has also been found recently by other workers in patients receiving phenytoin alone (Reynolds et al, 1976). The patients in the present study who showed improved control of major seizures with raised phenytoin dose were those with generalised activity in their EEG's; there was no overall effect in patients with temporal lobe epilepsy.

In assessing the effect of increased phenytoin therapy in these patients it should be taken into consideration that there was also an increase in phenobarbitone serum levels. While it seems likely that the increase in phenobarbitone serum concentrations was of less therapeutic importance than the increase in phenytoin serum concentrations, this finding is of interest in view of the fact that treatment of epilepsy with a combination of these drugs, which is often recommended (Schmidt and Wilder, 1968; Aird and Woodbury 1974) and widely practised (see Table 3.2), is based on a suggested synergistic action between these two drugs. The effect of phenytoin in increasing serum concentrations of phenobarbitone as found here may account for an apparent synergistic action. Conversely, a recent study (Leppik and Sherwin, 1977) has shown that an apparent potentiation of anticonvulsant activity against electroshock seizures

in rats when these drugs were administered simultaneously could be accounted for by the effect of phenobarbitone in increasing serum concentrations of phenytoin. The effect of phenytoin on metabolism of phenobarbitone is discussed further in Chapter 5.

In Chapter 3 it was found that comparison of serum phenytoin concentrations in patients receiving phenytoin alone and patients receiving phenytoin plus phenobarbitone did not show any significant effect of the administration of phenobarbitone. Because of the wide scatter of concentrations even in patients on the same dose it is possible that an effect might not be detected; and this trial was therefore designed also to examine the effect of phenobarbitone on the relationship between phenytoin dose and serum levels in individual patients. All the twenty patients in the trial were receiving either phenobarbitone or primidone, and comparison was made with five patients receiving phenytoin alone who also had their doses increased and the K_m and V_{max} values determined. Patients receiving phenytoin alone showed generally higher K_m values; there was no difference in V_{max} values between the two groups of patients. Also, the patients on whom the nomogram (Richens and Dunlop, 1975) was based, most of whom were receiving phenytoin alone, showed a higher mean K_m than the patients in the present study on combined therapy.

The finding of a lower K_m in the patients receiving phenobarbitone, either as the drug itself or as primidone, is somewhat surprising. There is evidence that phenobarbitone may act as a competitive inhibitor of phenytoin metabolism in vitro (Kutt and Verebely, 1970) and this would be expected to increase K_m . In addition, studies both of serum half-lives (Cucinell et al, 1963; Frey et al, 1968; Buchanan et al, 1969; Kristensen et al, 1969), and of metabolism by rat liver microsomes (Kutt and Fouts, 1971) have suggested that pre-treatment with phenobarbitone induces phenytoin metabolism; this would be expected to increase V_{max} , which was not found in the present study.

It appears that, with chronic administration of phenobarbitone and phenytoin, the inducing and inhibitory effects of phenobarbitone on phenytoin metabolism tend to cancel. As was discussed in Chapter 3, there have been a number of conflicting reports of a raising or lowering of phenytoin serum levels when phenobarbitone is administered but the general effect appears to be slight.

It might still be expected that phenobarbitone administration would raise the K_m and V_{max} for phenytoin metabolism, although serum levels are unaffected. However, it should be taken into consideration that, in this study, phenobarbitone serum levels increased as phenytoin dose and serum level were raised. Fig 4.7 shows the expected relationship between dose and serum concentration of phenytoin graphed as a reciprocal plot (Lineweaver - Burk), which is an alternative method to that shown in Fig 4.5 for determination of K_m and V_{max} . The effect of a competitive inhibitor such as phenobarbitone is shown by the interrupted line - K_m is increased while V_{max} is unaffected. The slope of the line, and hence K_m , is increased as inhibitor concentration increases; however, in this case the concentration of the inhibitor (phenobarbitone) does not stay constant. In this study the increase in serum phenytoin caused an increase in serum phenobarbitone, either due to inhibition of phenobarbitone metabolism or stimulation of conversion of primidone to phenobarbitone. This combined interaction of phenytoin and phenobarbitone might produce a non-linear relationship in the reciprocal plot as shown. The effect of this would be to produce apparently lower values of K_m and V_{max} . As was stated above, phenobarbitone may also act as an inducer of phenytoin metabolism and this would raise V_{max} . The inhibitory and inducing effects of phenobarbitone may cancel and produce no overall change in V_{max} .

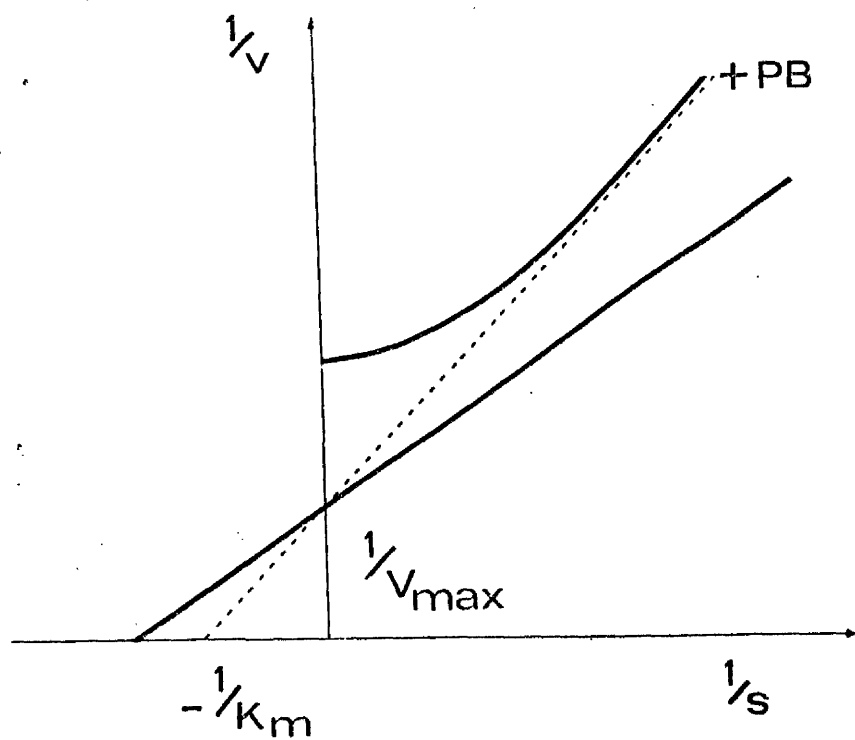


Fig 4.7

Possible kinetics of phenytoin metabolism in the presence of phenobarbitone. Simple competitive inhibition is shown by the interrupted line; a more complex interaction may produce a curve similar to that shown

It should be noted that, if administration of drugs such as phenobarbitone produce effects on phenytoin metabolism as described here, then not only might the nomogram not be valid but even determination of the individual K_m and V_{max} would not accurately predict the relationship between dose and serum concentration of phenytoin. It is, therefore, important to adjust the dose in small increments, with constant measurement of serum concentrations, or toxic effects due to high concentrations may result.

SUMMARY

1. A study has been conducted to assess the value of a nomogram proposed by Richens and Dunlop (1975) for predicting therapeutic doses of phenytoin. This nomogram attempted to predict the relationship between dose and serum concentration of phenytoin in individual patients from a single measured serum concentration.
2. Twenty poorly controlled epileptic patients receiving anticonvulsant therapy were investigated. All were receiving phenytoin and showed low serum concentrations of the drug.
3. Increase of serum concentrations of phenytoin to above 40 $\mu\text{mol/l}$ improved control of major but not minor seizures.
4. Phenobarbitone serum concentrations were found to increase as the phenytoin dose was raised.
5. The nomogram was found to be of only limited value in predicting serum concentrations in these patients. It is suggested that this may be because phenobarbitone alters the relationship between dose and serum concentration of phenytoin due to inhibition of phenytoin metabolism.

CHAPTER 4A

HOW DOES AGEING AFFECT THE RELATIONSHIP BETWEEN PHENYTOIN DOSE AND SERUM CONCENTRATION?

INTRODUCTION

Epilepsy is a common clinical problem in old age (Hildick-Smith, 1974), cerebrovascular disease and dementia of unknown aetiology being the most frequent causes (British Medical Journal, 1975). Good control of fits can usually be established with anticonvulsants and, since phenobarbitone is often regarded as a potentially toxic drug in the elderly, phenytoin is the anticonvulsant of choice.

In Chapter 3 it was shown that, in patients receiving the same dose of phenytoin, the serum concentration is significantly increased with age. The doses of phenytoin recommended for the elderly are generally lower than those used for younger patients (Exton-Smith and Windsor, 1971; Balme, 1977). This Chapter describes a study which was conducted to determine whether there is a rationale for reducing phenytoin doses in the elderly based on a reduced ability to metabolise the drug.

PATIENTS AND METHODS

Seventeen patients aged 67 to 96 years (3 male and 14 female) were studied. The cause of seizures was cerebral infarction in eight cases, 'senile dementia' in four, intracranial tumour in two and uncertain in two. In addition to phenytoin, three patients were also being treated with benzodiazepines, two with phenobarbitone and two with thioridazine.

Serum phenytoin was measured on blood samples taken between 0900 and 1100 hours. Single measurements were made on six patients, two measurements on two patients,

three on four patients, and four or more on four patients on different dose levels. All patients but one (two weeks only) had been taking phenytoin for three weeks before the first determination, and each dose level had been maintained for at least seven days before blood was taken.

RESULTS

Fig 4A.1 shows the relationship between phenytoin dose and serum concentration for the 42 samples obtained. No serum concentration was in the range 40 - 80 $\mu\text{mol/l}$ on any dose less than 300mg/day. Of 13 measurements on doses of 300mg/day, three were over 40 $\mu\text{mol/l}$. Four of five measurements on patients receiving 350mg/day or more were substantially higher (64 - 133 $\mu\text{mol/l}$) but there was no clear evidence of toxicity in any patient.

Fig 4A.2 shows a plot of dose against dose/mean serum concentration for four dose levels, of 150mg/day and over; the serum concentrations for lower doses, of 75 and 100mg/day, do not appear to fit the Michaelis-Menten relationship and have been excluded. The mean values obtained for V_{max} (375mg/day) and K_m (2.5 $\mu\text{g/l}$; 12 $\mu\text{mol/l}$) are very close to those which were previously found in Chapter 4 for younger patients on multiple drug therapy [V_{max} : mean 375mg/day, range 250 - 550mg/day; K_m : mean 2.2 $\mu\text{g/l}$ (8.8 $\mu\text{mol/l}$) range 1.4 - 4.8 $\mu\text{g/l}$ (5.6 - 19.2 $\mu\text{mol/l}$)], although they are somewhat lower than younger patients on single drug therapy with phenytoin. Fig 4A.3 shows the curve relating dose to serum concentration derived from the values for V_{max} and K_m in the elderly.

DISCUSSION

This study shows that, similar to findings in younger patients, doses of phenytoin of less than 300mg/day do not produce serum concentrations in the range 40 - 80 $\mu\text{mol/l}$ and even doses of 300mg/day do so relatively infrequently. It is of course possible that in old age increased sensitivity of the brain to drugs might be accompanied by a reduction in the lower limit of the therapeutic range,

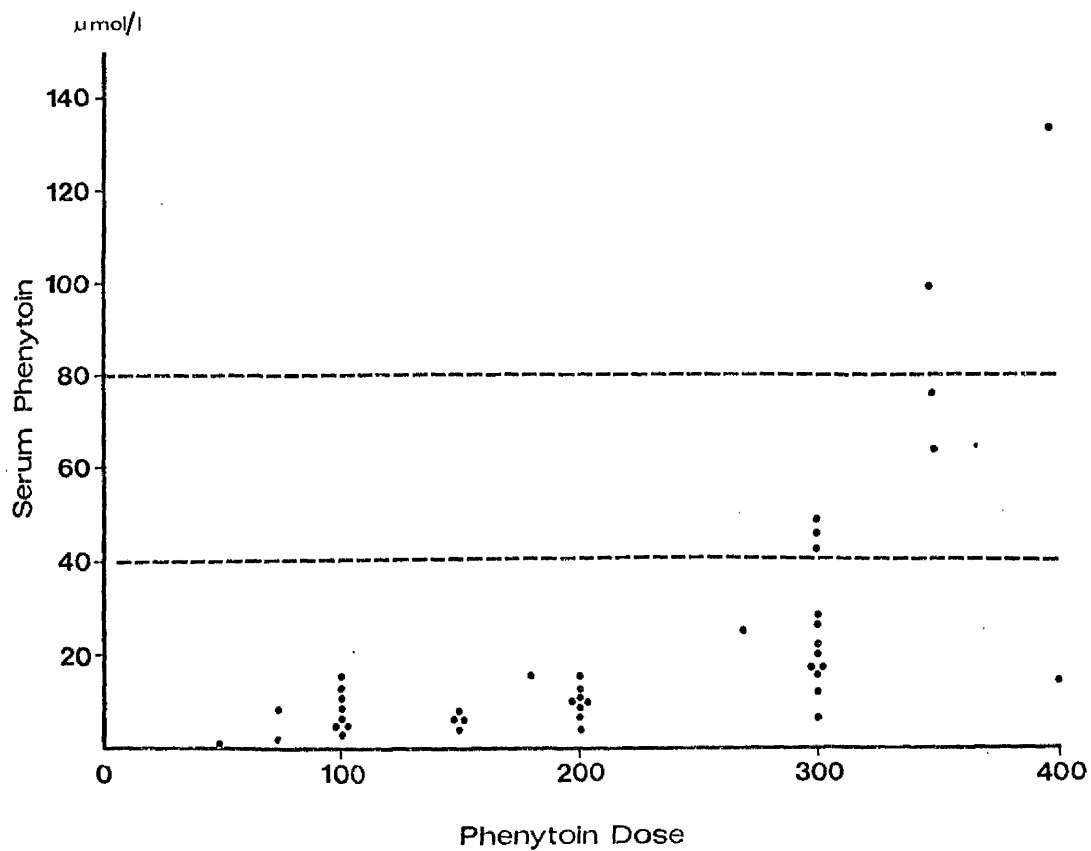


Fig 4A.1 Relationship between phenytoin dose and serum concentrations for 43 samples obtained at different dose levels from 17 geriatric patients

$$V_{\max} = 375 \text{ mg/day}$$

$$K_m = 2.52 \mu\text{g/ml}$$

$$= 10.8 \mu\text{mol/l}$$

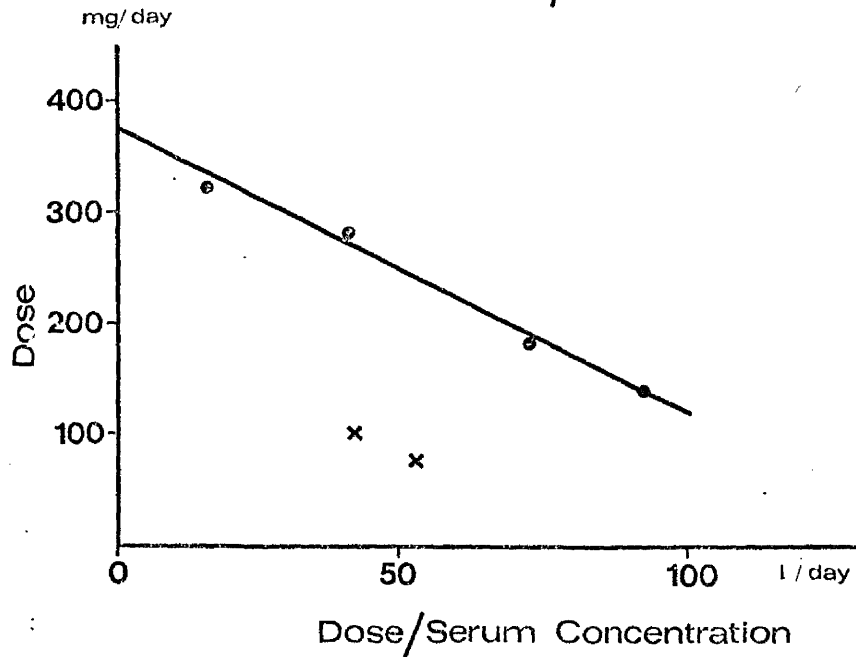


Fig 4A.2

Plot of phenytoin dose against dose/serum concentration for samples obtained from geriatric patients on doses of 150-350mg/day (•). Crosses represent doses of 100 and 75mg/day. This plot allows determination of V_{\max} (intercept on the y-axis) and K_m (the negative slope of the line)

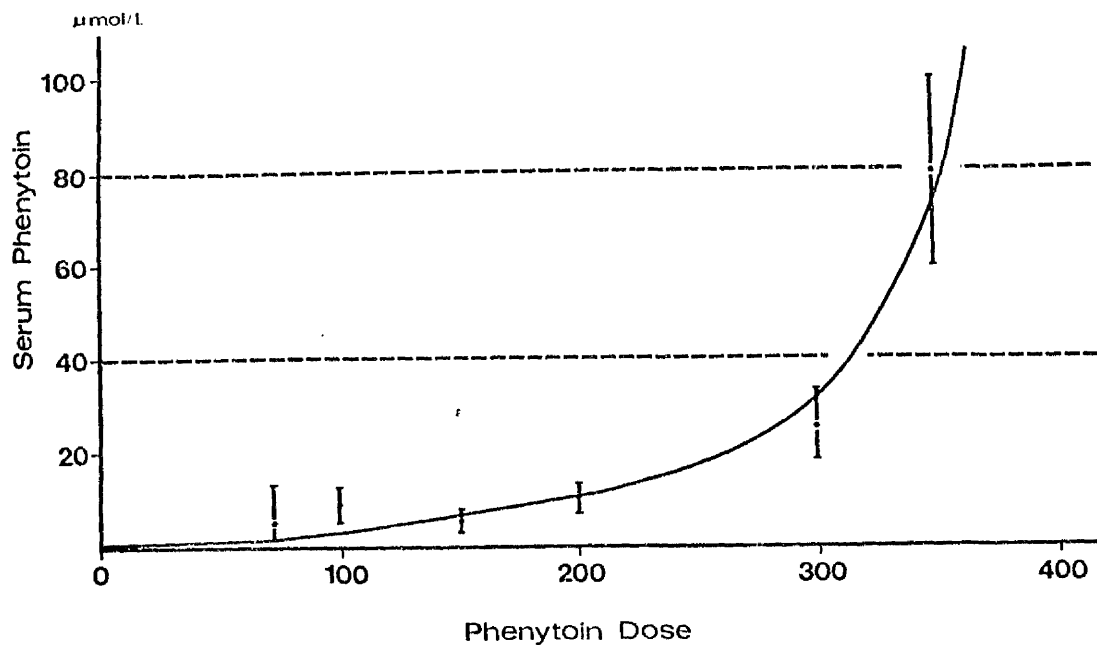


Fig 4A.3

Serum phenytoin concentrations (mean \pm SE) in relation to dose in the elderly. The curve has been derived from the values of V_{\max} and K_m obtained from the previous figure

but in the only instance of such a phenomenon at present documented, nitrazepam (Castleden et al, 1977), the increase in sensitivity was modest. In the present study none of the patients displayed symptoms of phenytoin toxicity even with fairly high serum concentrations. It must be concluded that the phenytoin doses of less than 300mg/day often advised for the elderly (Exton-Smith and Windsor, 1971; Balme, 1977) are likely to be inadequate. A reasonable maintenance dose would seem to be 300mg/day.

The data are consistent with the view that the kinetics of phenytoin metabolism are not substantially different in the elderly from those in younger patients. An age-related reduction in the hepatic metabolism of other drugs has been suggested for antipyrine (O'Malley et al, 1971; Vestal et al, 1975), phenylbutazone (O'Malley et al, 1971), amylobarbitone (Irvine et al, 1974), diazepam (Klotz et al, 1975) and propranolol (Castleden et al, 1975), but a wider scatter about the mean values is striking, and age is certainly not the main determinant of the rate of metabolism of any of these drugs.

Apart from reduced metabolism in the elderly, it is possible that there might be reduction in protein-binding of phenytoin. It would in fact be expected that a lowered degree of protein binding would produce an increased elimination of the drug, and it may be that there is a decreased capacity for drug metabolism in the elderly which could not be observed in this study because of this effect. The relationship between age and protein binding of phenytoin is discussed in Chapter 8, but it appears that the protein binding is reduced only if there is a low serum albumin level.

SUMMARY

1. Serum phenytoin concentrations were measured in 17 elderly patients, a total of 42 samples being obtained at different dose levels.
2. Therapeutic phenytoin concentrations were not produced by doses of less than 300mg/day. Determination of mean values for V_{max} and K_m showed little difference from values obtained for younger patients, suggesting that phenytoin metabolism is not greatly altered in old age.

CHAPTER 4B

HOW DOES PHENYTOIN METABOLISM ALTER WITH INCREASING DOSE?

INTRODUCTION

In Chapter 4 a non-linear relationship between dose and serum concentration of phenytoin has been described. This is probably due to saturation of the enzyme system involved in metabolism. The metabolism of phenytoin involves conversion in the liver to p-hydroxy-phenytoin (HPPH) which is conjugated with glucuronic acid before excretion in the liver (Noach et al, 1958). Since the non-linear relationship between dose and serum concentration makes it difficult to obtain therapeutic serum concentrations, a method of predicting serum concentrations is desirable. One method which has been proposed is the nomogram (Richens and Dunlop, 1975) which was described and evaluated in Chapter 4. Another possible method is the use of the HPPH/phenytoin ratio in a 24-hour urine sample after a single oral dose (Toseland and Albani, 1974); it was suggested that this gives an index of the steady state serum concentration that will be achieved with chronic therapy.

It has been shown that the urine HPPH/phenytoin ratio correlates negatively with the serum phenytoin concentration in a group of 'steady state' patients (Houghton and Richens, 1974). However, in that study no allowance was made for the possibility that the fraction of the phenytoin dose which is excreted as phenytoin plus HPPH may alter with increasing dose; for example, it has been suggested that the proportion of the dose excreted as HPPH may alter due to conversion of phenytoin to a secondary metabolite (Eadie et al, 1976). This possibility should perhaps be taken into consideration in describing the relationship between the HPPH/phenytoin ratio in urine and the serum phenytoin concentration. This Chapter describes an initial study which was carried out to determine how the

proportion of the phenytoin dose excreted as HPPH and phenytoin alters with increasing serum concentration.

PATIENTS AND METHODS

24-hour urine samples were obtained from 12 epileptic in-patients on chronic phenytoin therapy. All were in addition receiving either phenobarbitone or primidone. Completeness of urine collection was determined by measuring creatinine production. Concentrations of phenytoin and HPPH in urine were determined by GLC, the HPPH conjugate being first hydrolysed with glucuronidase (see Chapter 2).

Blood samples were also obtained from these patients for measurement of serum phenytoin.

RESULTS

Fig 4A.1a shows the percentage recovery of the phenytoin dose (free acid) as HPPH correlated against the serum phenytoin concentrations in these patients. One patient showed a very low HPPH recovery of 22% of the dose (this result proving repeatable when a second 24-hour urine sample was obtained); this patient was suffering from psoriasis. Excluding this patient, there was a negative trend between the output of HPPH and the serum phenytoin concentration but this did not reach significance. The negative trend in HPPH production as the serum concentration is increased can be explained at least partly by the increased excretion of phenytoin; correlating the percentage of the dose excreted as phenytoin with the serum concentration, a positive relationship was found, as would be expected. A significant negative linear relationship was found between the HPPH/phenytoin concentration ratio in urine and the serum phenytoin concentration ($r = 0.619$, $p < 0.05$) as shown in Fig 4A.1b

DISCUSSION

A negative relationship was found between the HPPH/phenytoin ratio in urine and the serum phenytoin concentration. This is in line with the hypothesis that the hydroxylation of phenytoin by hepatic microsomal enzymes is a saturable

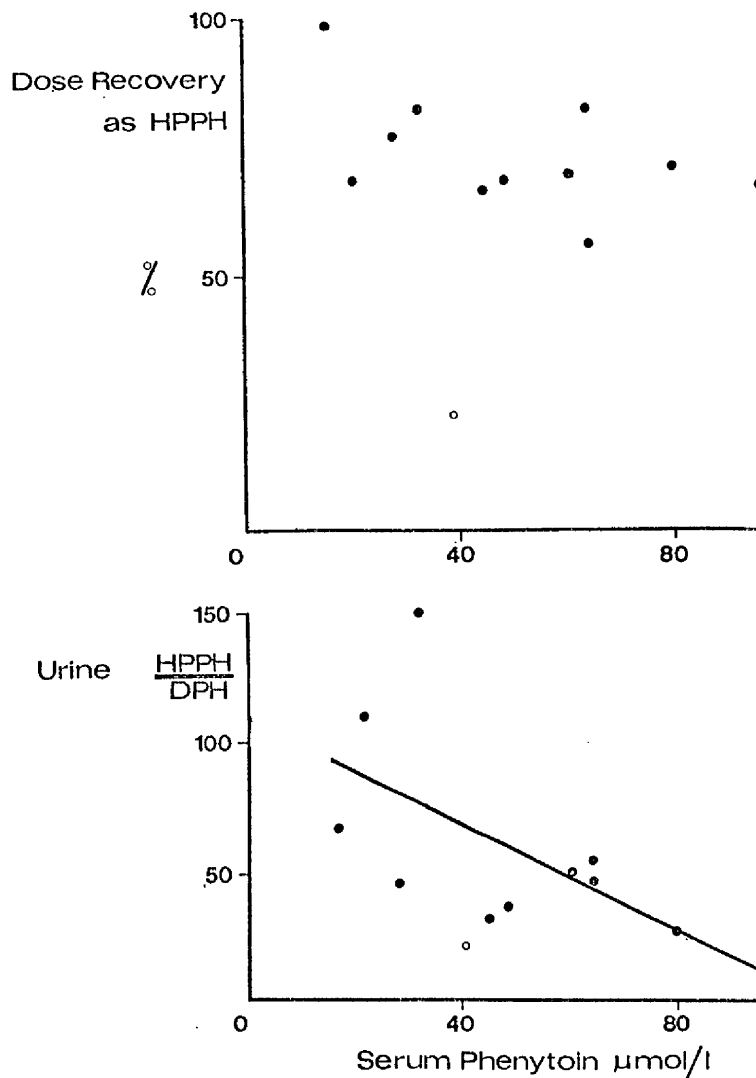


Fig 4B.1a Percentage of phenytoin dose recovered in the urine as HPPH in relation to serum phenytoin concentration, for 12 patients in steady-state. A patient who was suffering from psoriasis is shown by an open circle

Fig 4B.1b The HPPH:phenytoin concentration ratio in the urine plotted against serum phenytoin concentration. A significant negative relationship ($p < 0.05$) is shown

process (Arnold and Gerber, 1970). The proportion of the dose recovered in the urine as HPPH plus phenytoin was approximately constant with increasing serum concentration; this suggests that no significant fraction of the dose is converted to a metabolite other than HPPH. A mean of 72% of the phenytoin dose was recovered as HPPH, which is in line with a single dose study in which about 70% of the dose was recovered as HPPH (Chang and Glazco, 1970).

One patient showed a considerably lower excretion of HPPH in the urine than other patients. This patient suffered from psoriasis, a skin disease with which there has been reported to be associated a malabsorption in the small intestine which causes increased excretion of faecal fat and of D-xylose (Shuster et al, 1967). It seems unlikely that this patient showed a defect in absorption of phenytoin, since serum levels on 350mg daily showed a reasonably high level of $64 \mu\text{mol/l}$. In addition, the patient was receiving primidone and serum levels and urinary excretion of this drug and its metabolites were normal. It is more likely that there was reduced reabsorption of HPPH - the metabolite is released into the bile after formation in the liver and absorbed in the small intestine before excretion by the kidney (Noach et al, 1958). Measurement of the HPPH/phenytoin ratio to predict serum phenytoin concentrations would be expected to give an erroneous result in this patient.

In conclusion, the urinary excretion of HPPH and phenytoin normally accounts for most of the dose even in patients showing high serum concentrations of phenytoin. However, it is possible that some patients may show reduced HPPH production due to reduced reabsorption in the small intestine. If this effect is common then it may produce errors in the use of the urine HPPH/phenytoin ratio (Toseland and Albani, 1974) as a method to predict serum phenytoin concentrations.

SUMMARY

1. Excretion of phenytoin and its major metabolite (HPPH) have been measured in 24-hour urine samples obtained from 12 patients on chronic phenytoin therapy.

2. There was a significant negative correlation between the HPPH/phenytoin ratio and the serum phenytoin concentration. There was no evidence that the percentage of the phenytoin dose excreted as the drug itself plus HPPH is altered with increasing dose.
3. A patient suffering from psoriasis showed reduced excretion of HPPH. It is suggested that this may be due to reduced reabsorption of the metabolite from the small intestine.

CHAPTER 5

PRIMIDONE METABOLISM AND THE EFFECTS OF PHENYTOIN UPON IT

INTRODUCTION

Primidone (Mysoline R) is a desoxybarbiturate which was reported to be clinically effective by Handley and Stewart in 1952 and is now widely used in the treatment of epilepsy. Primidone has two major metabolites both in animals and man - phenobarbitone and phenylethymalonamide (PEMA) (Bogue et al, 1956; Butler and Waddell, 1956). The structure of primidone and its metabolites are shown in Fig 5.1. Phenobarbitone has been employed as an anticonvulsant since 1912 and primidone exerts its anticonvulsant activity at least partly via oxidation to phenobarbitone. In addition, PEMA has been shown to have anticonvulsant activity in rats (Baumel et al, 1972). Since primidone is converted in vivo to two active metabolites, variations in the metabolism of the drug between different individuals may be of therapeutic importance. In Chapter 4 it was shown that phenytoin may increase serum concentrations of phenobarbitone in patients receiving either phenobarbitone or primidone and other workers have suggested that phenytoin increases serum concentrations of phenobarbitone in patients receiving primidone (Wilson and Wilkinson, 1973; Fincham et al, 1974; Reynolds et al, 1975). In this Chapter further observations on the effects of phenytoin on primidone metabolism are described, particularly with reference to the metabolism of primidone to PEMA which has been little studied previously. Serum concentrations of the drug and its metabolites have been measured in patients receiving primidone alone and patients receiving primidone plus phenytoin; in addition, concentrations in urine have been measured to determine how accurately serum concentrations reflect the metabolism. Primidone has a fairly short half-life of about six hours (Gallagher and Baumel, 1972) and therefore serum concentrations of primidone are usually measured shortly after

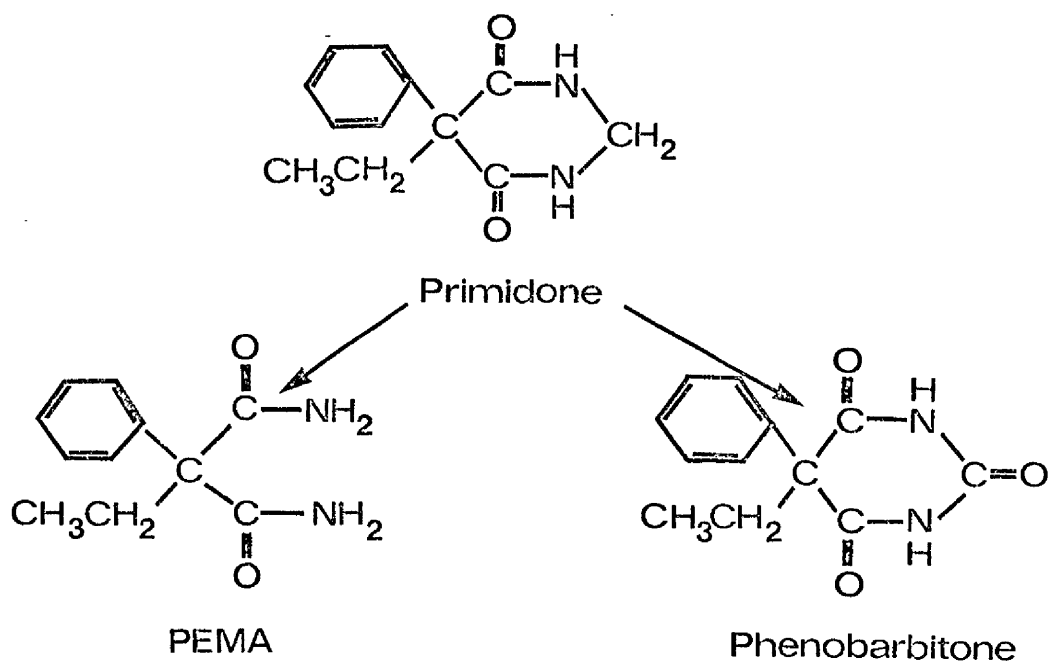


Fig 5.1 Structures of primidone and its metabolites

the previous dose. However, it would be expected that serum concentrations measured in this way would depend mainly on the peak serum concentration obtained with this previous dose and would not reflect to any great extent the rate of metabolism of primidone. Therefore, in this study serum concentrations of primidone have also been measured some considerable time (12 hours) after the previous dose.

PATIENTS AND METHODS

The subjects studied were 58 male and 36 female patients, with ages ranging from 16 to 66 years. Of these, 59 were outpatients attending the epilepsy clinic at the Institute of Neurological Sciences, Glasgow, and 35 were inpatients at the Epilepsy Centre, Bridge of Weir. All patients were receiving primidone, in doses ranging from 250-1500mg daily, and 55 patients were also receiving phenytoin.

Blood samples for anticonvulsant analysis were obtained from 60 patients between 2 and 4 hours after the last drug dose and from the other 34 patients blood samples were obtained 12 hours after the last drug dose. Concentrations of phenytoin and primidone were determined by gas chromatography (see Appendix).

Urine was collected over a period of 24 hours from 10 of these patients of whom 6 were receiving primidone plus phenytoin and 4 were receiving primidone alone. Completeness of urine collection was assessed by measuring 24-hour creatinine production. Drug analyses were performed as in serum but it was necessary to dilute urine containing high concentrations of drug. Any drug or metabolite which might exist as the glucuronide derivative rather than the free drug was measured by hydrolysing the conjugates with glucuronidase.

RESULTS

Serum Concentrations of Phenobarbitone

The serum concentrations of phenobarbitone in relation to the dose of primidone are shown in Fig 5.2 for 19 patients receiving primidone alone and 52 patients receiving primidone

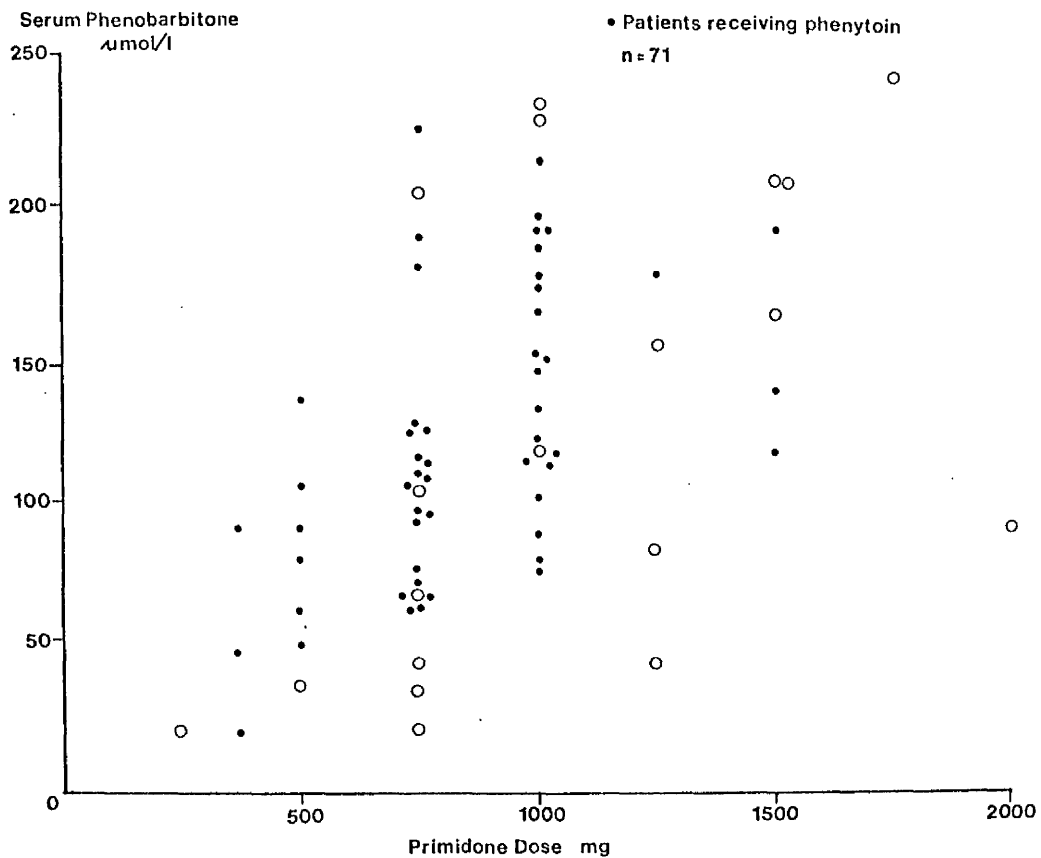


Fig 5.2

Serum concentrations of derived phenobarbitone in patients receiving primidone. 19 patients were receiving primidone alone and 52 were receiving phenytoin also

and phenytoin (no patients receiving other drugs have been included). There were significant correlations between dose and serum concentration for both groups of patients (Table 5.1). Patients receiving phenytoin showed slightly higher concentrations. Although there is little difference between the slopes of the lines, it is noticeable that in several patients receiving primidone alone showed very low serum phenobarbitone concentrations in relation to dose.

Serum Concentrations of Primidone

The serum concentrations of primidone in relation to dose are shown in Fig 5.3 for 52 patients from whom blood samples were obtained 2-4 hours after the previous drug dose (15 were receiving primidone alone) and 29 patients from whom blood samples were obtained 12 hours after the previous dose (5 were receiving primidone alone). For the former group of patients there were significant correlations between dose and serum concentrations (Table 5.2). Considering only the 24 patients who were receiving 300mg phenytoin daily, a higher correlation was found ($r = 0.693$) than when all 34 patients receiving phenytoin were considered ($r = 0.566$). There was some evidence, at least in the patients receiving phenytoin, that increase of the primidone dose above 1000mg did not produce the expected rise in primidone serum concentrations. Evidence for non-linearity was also found from measurement of serum concentrations of primidone 12 hours after the previous dose - particularly for patients receiving primidone alone, the serum concentrations were not increased with increasing dose.

The Phenobarbitone/Primidone Ratio

The patients receiving primidone alone showed slightly elevated primidone serum concentrations and in a number of cases lower phenobarbitone serum concentrations than patients receiving phenytoin; this would suggest that the phenobarbitone concentrations are higher at least in relation to primidone concentrations in patients receiving phenytoin. This was confirmed when the phenobarbitone/primidone ratios were compared, for concentrations measured 2-4 hours after

Y	X	ON DPH	NO OF PATIENTS	m	b	r	P
Serum PB $\mu\text{mol/l}$	Primidone dose mg	-	19	0.0901	26.4	0.500	0.05
Serum PB $\mu\text{mol/l}$	Primidone dose mg	+	52	0.0934	41.8	0.524	0.001
Serum Primidone $\mu\text{mol/l}$	Primidone dose mg	-	15	0.0433	11.3	0.755	0.001
Serum Primidone $\mu\text{mol/l}$	Primidone dose mg	+	36	0.0330	11.5	0.566	0.001
Serum PB/Primidone (log)	Serum DPH (log)	+	22*	0.178	0.208	0.399	0.1
Serum PEMA $\mu\text{mol/l}$	Serum Primidone $\mu\text{mol/l}$	-	9	0.752	-1.9	0.838	0.01
Serum PEMA $\mu\text{mol/l}$	Serum Primidone $\mu\text{mol/l}$	+	31	0.749	1.9	0.615	0.001
Serum PEMA/Primidone	Primidone dose mg	-	9	2.33×10^{-4}	0.297	0.286	-
Serum PEMA/Primidone	Primidone dose mg	+	31	6.72×10^{-4}	0.513	0.305	0.1

* Only the 22 patients receiving 300mg DPH daily were considered

Table 5.1

Relationships Between Serum Concentrations of Primidone and its Metabolites and Primidone Dose, Serum Primidone Concentration and Serum Phenytoin Concentration

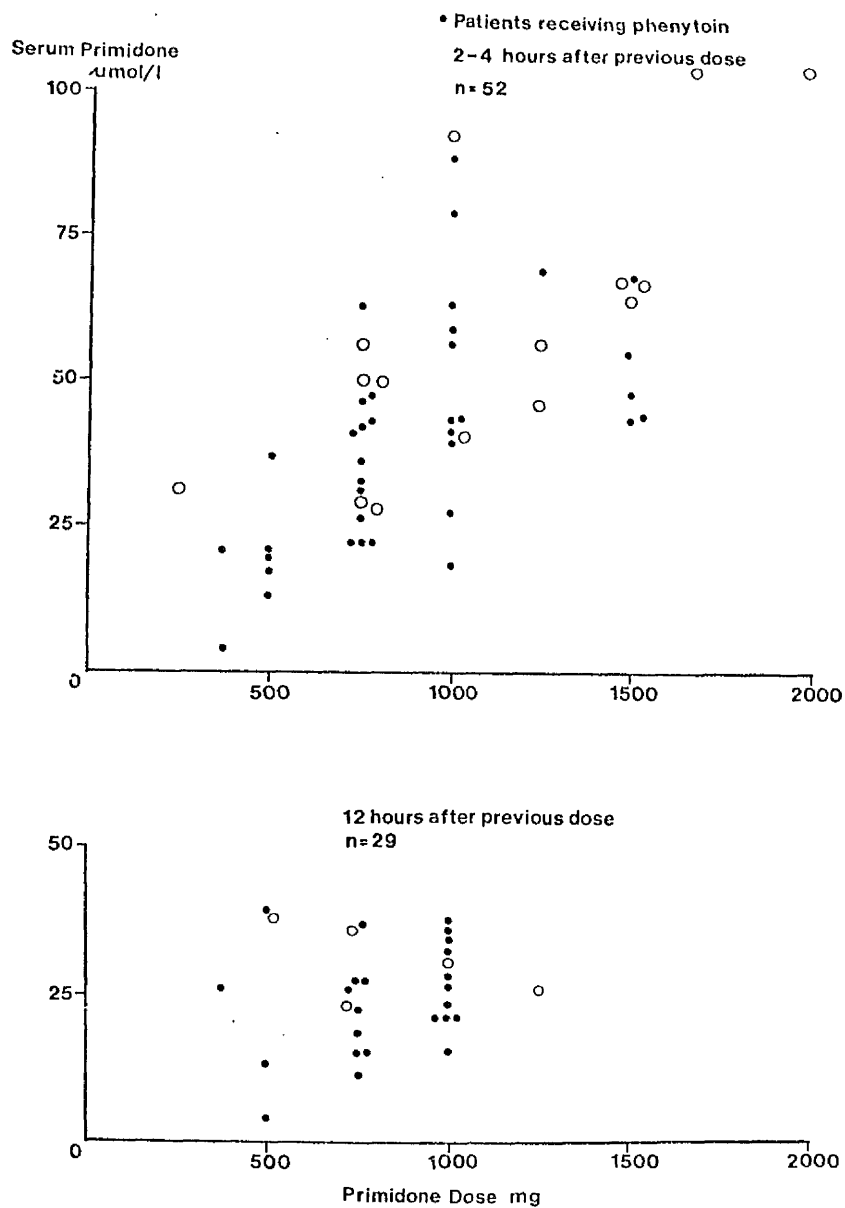


Fig 5.3

Serum concentrations of primidone in samples obtained from 52 patients 2-4 hours after the previous dose and from 29 patients 12 hours after the previous dose of the drug. Patients were either receiving primidone alone or combined therapy with phenytoin

the dose; 31 patients on phenytoin showed a significantly higher ratio than 14 patients receiving primidone alone ($t = 4.513$, $p < 0.001$, comparing the log values for the ratio). In the 22 patients receiving 300mg phenytoin daily there was a trend towards higher phenobarbitone/primidone ratios with increasing serum concentrations of phenytoin and this just failed to reach significance (Table 5.1) (correlating the log values for the ratio and for serum phenytoin concentration).

Serum Concentrations of PEMA

Serum concentrations of PEMA were measured in 40 of the patients receiving primidone, 31 of whom were receiving phenytoin also. The relationship between serum concentrations of primidone and PEMA is shown in Fig 5.4 and Table 5.1. There was a significant correlation between the serum concentrations both for patients receiving phenytoin and patients receiving primidone alone; patients receiving phenytoin showed generally higher levels of PEMA in relation to primidone, although the slopes of the lines were the same. Comparing the PEMA/primidone ratios in patients receiving phenytoin to those in patients receiving primidone, a significant difference was indeed found ($t = 3.04$, $p < 0.005$). There was, in addition, a possible trend towards a higher PEMA/primidone ratio in patients receiving higher doses, but this did not reach significance (Table 5.1).

Urinary Excretion of Primidone and its Metabolites

Measurement of excretion of primidone and its metabolites was carried out in 24-hour urine samples obtained from 10 patients receiving primidone. Measurements were carried out by gas chromatography as before, but any drug or metabolite which might exist as a glucuronide conjugate was first hydrolysed enzymatically (see Chapter 2 and Appendix); no evidence was found, however, for significant conjugation of primidone or PEMA in urine, comparing the free with the total concentrations.

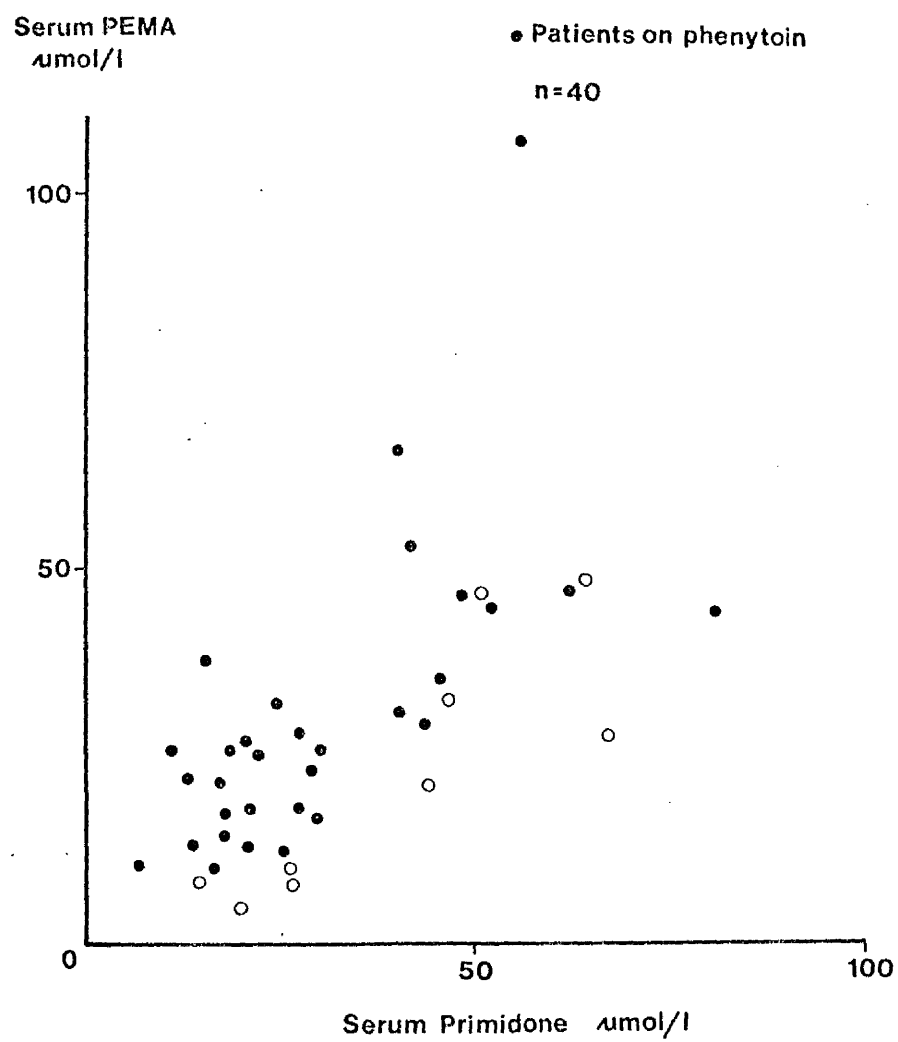


Fig 5.4

Serum concentrations of PEMA plotted against primidone concentrations for 40 patients receiving primidone, 31 of whom were also receiving phenytoin

The percentage of primidone dose which was recovered in urine as the drug itself or as its metabolites was $72.5\% \pm 12.3$ SD. In line with the findings from serum concentrations, the PEMA : primidone ratio and phenobarbitone : primidone ratio were higher in patients receiving phenobarbitone - in four patients receiving primidone alone the PEMA : primidone and phenobarbitone : primidone ratios showed means of 0.58 ± 0.41 SD and 1.00 ± 0.04 SD respectively and for patients receiving phenytoin the means were 0.98 ± 0.08 SD and 0.21 ± 0.08 SD respectively. There were reasonably high correlations between these ratios in serum and urine, $r = 0.781$ for the PEMA : primidone ratio and $r = 0.712$ for the phenobarbitone : primidone ratio (note that the serum concentrations of primidone and its metabolites were measured 12 hours after the previous dose in these patients). The actual values for the PEMA : primidone ratios in serum and urine were almost identical but the phenobarbitone : primidone ratio in serum was twelve times that in urine.

For comparison, measurements of phenobarbitone concentrations were carried out in 24-hour urine samples obtained from 5 patients receiving phenobarbitone (all of these were also receiving phenytoin). A mean fraction of 0.41 ± 0.11 SD of the dose was recovered as phenobarbitone in the urine. Using this value it was possible to calculate approximately how much of the primidone dose is converted to each of its metabolites - in the 10 patients studied, $18\% \pm 7$ SD of the dose recovered in the urine was phenobarbitone (phenobarbitone itself plus its hydroxylated metabolite) and $35\% \pm 10$ SD was PEMA. In the only other study of urinary excretion of primidone and both its metabolites, in rabbits, the extents of conversion of primidone to phenobarbitone and PEMA were 25% and 65% respectively (Fujimoto et al, 1968)

DISCUSSION

Many patients receive combined therapy of phenytoin and primidone (see Chapter 3) and interactions between these drugs may be of therapeutic significance. The results in this study suggest that phenytoin may have an effect on the

metabolism of primidone which causes higher serum concentrations of derived phenobarbitone. This effect may be related to the serum concentrations of phenytoin, since in the patients receiving 300mg phenytoin daily there was a positive trend which almost reached significance between the phenobarbitone : primidone ratio and the serum phenytoin concentration. The latter finding was described previously (Reynolds et al, 1975) and it was suggested that the effect of phenytoin may be an inhibition of the metabolism of phenobarbitone. Other workers have shown that an increased phenobarbitone : primidone ratio in patients on phenytoin (Fincham et al, 1974; Callaghan et al, 1977) and have suggested that phenytoin induces the liver enzyme system responsible for the oxidation of primidone to phenobarbitone. An inhibitory effect of phenytoin on phenobarbitone metabolism, as at least a contributory factor, appears likely since a similar greater effect of phenytoin on phenobarbitone concentrations was described in Chapter 3 in patients receiving phenobarbitone rather than primidone. Also, in the study described in Chapter 4, phenobarbitone concentrations increased in individual patients receiving either phenobarbitone or primidone, in whom the phenytoin dose and serum concentrations were increased.

Enzyme induction may nonetheless be important in the metabolism of primidone. There appears to be a non-linear relationship between dose and serum concentrations of primidone which might be explained as a self-induction of its own metabolism, presumably mainly to PEMA rather than phenobarbitone. This is confirmed by measurement of serum concentrations of PEMA and it appears that, in addition, phenytoin may induce primidone metabolism to PEMA. Auto-induction by primidone of its metabolism has been found by other workers during acute administration (Gallagher et al, 1972) but in this case the drug appeared to induce its own metabolism to phenobarbitone rather than to PEMA ; phenobarbitone did not appear in the serum until 24 to 96 hours after the initiation of primidone therapy whereas PEMA appeared within 24 hours. However, a patient in whom the appearance of phenobarbitone was delayed for 96 hours showed only low levels of PEMA within this period. A study of

primidone metabolism in perfused rat liver (Alvin et al, 1975) showed that pretreatment with phenobarbitone or primidone induced metabolism both to phenobarbitone and PEMA. It appears from the present study that, in patients on chronic therapy, the inducing effect of primidone on its own metabolism to PEMA, and possibly also to phenobarbitone, may increase with increasing dose of the drug. It has previously been suggested that there is a linear relationship between dose and serum concentrations of primidone (Gallagher and Baumel, 1972), but the evidence in the present study suggests that this may not be entirely true and another recent study has similarly found the serum levels of primidone levelled off at about 30 $\mu\text{mol/l}$ in adult patients (Dekaban et al. 1974).

The possible inducing effect of primidone on its own metabolism and the effects of phenytoin may be of clinical importance and this depends upon the relative therapeutic effects of primidone and its metabolites. It has been found that doses of primidone required to obtain an anti-convulsant effect comparable to a given dose of phenobarbitone result in comparable serum concentrations of phenobarbitone (Bogan and Smith, 1968). Olesen and Dam (1967) found no difference in the clinical effectiveness of the two anticonvulsants when similar serum concentrations of phenobarbitone were obtained and they suggested that the anticonvulsant activity of primidone is simply due to the derived phenobarbitone. If this were the case it might be expected that combined therapy of phenytoin and primidone would produce a synergistic effect (as may happen with phenytoin and phenobarbitone) due to the action of phenytoin in increasing serum concentrations of phenobarbitone. However, studies in rats have suggested that primidone itself probably does have some anticonvulsant activity since the drug is more effective than phenobarbitone in raising seizure threshold with comparable or even lower phenobarbitone serum levels (Gallagher et al, 1970). PEMA has also been shown to have anticonvulsant activity in rats, even at a low dose at which phenobarbitone would have no effect (Baumel et al, 1972). If primidone has a greater anticonvulsant activity

than PEMA and phenobarbitone metabolites, it would be expected that administration of phenytoin will lower the therapeutic effect of the drug; also, since the drug induces its own metabolism there may be little value in high doses.

In the present study it has been shown that the PEMA/primidone ratio is increased in patients receiving phenytoin and it is also suggested that the ratio increases with increasing primidone dose. This would suggest that the metabolism of primidone to PEMA is dependent on the combined doses of enzyme-inducing drugs that the patient is receiving. In Chapter 12 a method for calculating 'Drug Units' according to the potential enzyme-inducing effect of a given drug regime is described; a significant positive relationship is found between the PEMA/primidone ratio and the Drug Units.

SUMMARY

1. Serum concentrations of primidone and its two metabolites (phenobarbitone and PEMA) have been measured in patients receiving either primidone alone or primidone plus phenytoin.
2. There was no significant difference between the mean concentrations of phenobarbitone for patients receiving primidone alone and for patients receiving primidone plus phenytoin. However, some patients on primidone alone showed unexpectedly low phenobarbitone concentrations. There may be an effect of phenytoin in inhibiting metabolism of phenobarbitone.
3. There was some evidence for non-linearity in the relationship between primidone dose and serum concentration, particularly in samples obtained some time after the previous dose. This may be explained by an inducing effect of primidone on its metabolism to PEMA.

4. There appeared to be an inducing effect of phenytoin on metabolism of primidone to PEMA. The PEMA/primidone ratio in serum was significantly higher ($p < 0.005$) in patients receiving phenytoin than in patients receiving primidone alone.
5. Concentrations of primidone and its metabolites were measured in 24-hour urine samples obtained from 10 patients. Evidence was found that the PEMA/primidone ratio and phenobarbitone/primidone ratio in serum were accurate reflections of the relative concentrations in urine.
6. In conclusion, administration of phenytoin to patients receiving primidone may increase serum concentrations of PEMA and possibly also of phenobarbitone, and decrease serum concentrations of primidone. This may be of therapeutic importance depending on the relative anticonvulsant activities of primidone and its metabolites.

CHAPTER 6

HOW DOES THE THERAPEUTIC EFFECT OF CLONAZEPAM CORRELATE WITH SERUM LEVELS AND DO DRUG INTERACTIONS ALTER THE EFFECTIVENESS OF THE DRUG?

"But I was thinking of a plan
To dye one's whiskers green
Then always use so large a fan
That they could not be seen"

- The White Knight in
"Alice's Adventures Through the
Looking Glass" by Lewis Carroll

INTRODUCTION

Chapters 4 and 5 have shown that drug interactions may affect serum levels and hence presumably the therapeutic response to the commonly used anticonvulsant drugs phenytoin, phenobarbitone and primidone. It is particularly important to investigate the possibility of drug interactions in assessing the value of new anticonvulsants, when these are given to patients already receiving drug therapy.

Clonazepam, 5-(2-chlorophenyl)-1,3 dihydro-7-nitro-2H-benzodiazepine-2-one (Rivotril^R) (Fig 6.1), is a new benzodiazepine anticonvulsant recently introduced into Britain and America. It was shown to be a more effective intravenous anticonvulsant than its benzodiazepine analogues diazepam and nitrazepam (Gastaut, 1971) and subsequent studies have shown that clonazepam is effective against a wide range of seizures (Browne and Penry, 1973; Lund, 1973; Scollo-Lavizzari et al, 1974).

However, there has been a lack of controlled trials of clonazepam and the value of the drug has not been clearly shown. The effect of simultaneous administration of other anticonvulsant drugs has not been investigated and a therapeutic range of serum concentrations of clonazepam has not been established. This Chapter describes the

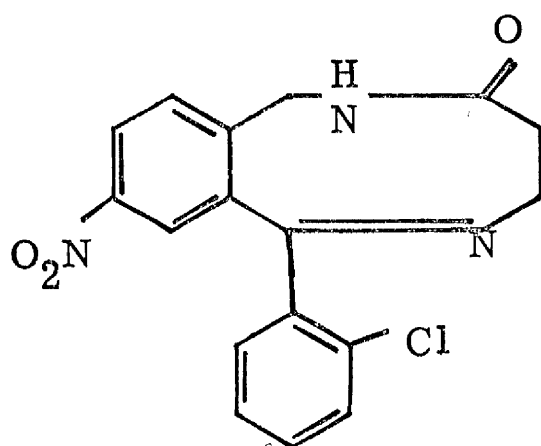


Fig 6.1 Chemical structure of clonazepam

effect of clonazepam in patients with various forms of epilepsy and receiving different anticonvulsant therapies and also the relationship of serum concentration to seizure frequency. The effects of clonazepam upon serum concentrations of other anticonvulsants have also been studied.

Both a double-blind trial and an open trial have been conducted to assess the value of clonazepam therapy. The double-blind trial extended over a period of nine weeks and the patients received a fixed dose of the drug; the patients were later followed up for a period of 21 months. The open trial extended over a period of 22 months. Nearly all the patients were receiving, in addition to clonazepam, one or more of the standard anticonvulsants.

PATIENTS AND METHODS

PATIENTS

Double-blind trial against placebo : Thirty patients, 22 male and 8 female, aged 11 - 40 years (mean 26 years) were studied. Fifteen patients had myoclonic epilepsy (myoclonic jerks and tonic-clonic seizures), four suffered from atypical absences and eleven patients had temporal lobe epilepsy. Twenty-five patients were receiving various combinations of phenytoin, phenobarbitone and primidone and five patients with myoclonic epilepsy were not receiving any medication. Details of the concurrently used anticonvulsant drugs are given in Table 6.1. Twenty were inpatients in a residential centre and ten were treated as outpatients.

Open trial : The 36 patients in the open trial of clonazepam, 19 male and 17 female, aged 11 - 44 years (mean 27 years), were treated as outpatients. Seven patients had myoclonic epilepsy, seven had photosensitive epilepsy, six had generalised tonic-clonic seizures and sixteen had temporal lobe epilepsy. All

Type of Epilepsy	Number of Patients	Phenytoin	Phenobarbitone	Primidone	Carbamazepine
Myoclonic	15	7	4	4	4
Atypical Absences	4	4	1	1	-
Temporal Lobe	11	10	6	5	5

Table 6.1
Concurrently used anticonvulsants in the double-blind trial
of clonazepam (30 patients)

patients were receiving various combinations of phenytoin, phenobarbitone and primidone; the patients with myoclonic and with photosensitive epilepsy were generally receiving low doses of single drugs whereas those with tonic-clonic and temporal lobe epilepsy were generally receiving combined therapy of high doses of drugs.

METHODS

In both trials clonazepam was added to the existing anti-convulsant regime. In the double-blind trial the initial dose of clonazepam was 1mg/day, increasing every third day by 1mg/day to 3mg/day (1mg morning and 2mg at night). Matched placebo tablets were used. At the end of 4-6 weeks the first tablets were reduced gradually while the second unknown tablet was introduced.

In the open trial, the clonazepam dose was also increased to an initial dose of 3mg/day, and the dose later increased or decreased as required. Those patients who benefitted during the double-blind trial were also managed in the same way. All outpatients recorded the frequency of their seizures and side-effects on a chart. Seizures and side-effects in inpatients were recorded by the nursing staff.

Blood samples for measurement of anticonvulsants were taken before and during clonazepam treatment. In addition to measurement of phenytoin, phenobarbitone and primidone by gas chromatography, clonazepam was measured by a radioimmunoassay technique (see Appendix) in 19 patients in the double-blind trial and 8 patients in the open trial on 3mg clonazepam daily. In addition, clonazepam levels were measured in 18 of these patients after one year of treatment, when they were receiving various doses of the drug. It was not possible to measure clonazepam in patients receiving other benzodiazepines such as diazepam or nitrazepam since they interfered with the assay.

RESULTS

Seizure Frequency

The effect of clonazepam on seizure frequency for the patients in the double-blind trial, both during the trial and after 21 months of therapy, is shown in Table 6.2. In the fifteen patients with myoclonic jerks, twelve of whom also had major (tonic-clonic) seizures, clonazepam caused suppression of jerks in twelve patients and 75% reduction in the other three; major seizures ceased to occur in eight patients and were reduced by 50% in the other four. The effectiveness of clonazepam therapy in the patients who improved was the same after 21 months as during the trial, but in four cases the clonazepam was increased to maintain effectiveness, doses of up to 12mg daily (one patient) being given without side-effects. It was possible to reduce or stop other anti-convulsants in four patients.

Four patients in the double-blind trial had temporal lobe epilepsy with psychomotor attacks and major seizures. There was no effect of clonazepam on the minor seizures although there was a reduction in major seizures in the four patients affected. After 21 months only two patients were still being treated with clonazepam.

For the patients in the open trial, the effect of clonazepam on seizure control after 22 months is shown in Table 6.3. The drug was again found to be effective in myoclonic attacks and major seizures. The drug was also highly effective in photosensitive epilepsy, with six patients showing abolition of seizures and the seventh showing marked improvement.

The sixteen patients in the open trial with temporal lobe epilepsy again showed little improvement on clonazepam. Within a year the drug was withdrawn in seven patients, in two patients because seizures increased and in five patients because they showed no improvement and suffered from side-effects.

Type of Epilepsy	Number of Patients	Trial Result			After 21 Months			
		100%	75%	50%	No Change	100%	75%	50%
Myoclonic	3	3				3		
Myoclonic + Tonic-Clonic								
Jerks	12	9	3			8*	3	
Tonic-clonic		8		4		7		4
Atypical Absences	4	3			11		2	2
Temporal Lobe								
Focal	11				11		2	
Tonic-clonic			4		7	2		9

Table 6.2

The effect of treatment with clonazepam in 30 patients during the double-blind trial and after follow-up for 21 months. In those patients who did not benefit, the drug was withdrawn.

* One died after 6 months - unrelated cause

Type of Epilepsy	Number of Patients	Improvement in Fit Frequency			Clonazepam Withdrawn
		100%	75%	50%	
Myoclonic + Tonic-clonic	7	7			
Photosensitive	7	6	1		
Generalised Tonic-clonic	6	2		2	2
Temporal Lobe	16			9	7

Table 6.3

The effect of treatment with clonazepam in 36 patients in the open trial. In the patients who did not benefit, the drug was withdrawn.

Six patients had generalised epilepsy with only tonic-clonic seizures. Clonazepam was somewhat ineffective and was stopped in two patients, in one case because an increase in seizure frequency and in the other due to side-effects.

Side Effects

The main side-effect with clonazepam was drowsiness (67% of patients receiving 3mg/day), especially during the introduction of the drug. After two to three weeks only six patients receiving 3mg of clonazepam (all in the open trial) continued to complain of drowsiness. These six patients were also ataxic and clonazepam was reduced and then stopped. None of the patients who continued to receive 3mg clonazepam for 21 or 22 months (45 patients) complained of any side-effects at the end of this time, even the four patients in whom doses were increased above 6mg to up to 12mg/daily (one patient).

Electroencephalogram (EEG)

Ten of the patients with myoclonic epilepsy showed generalised polyspike-wave seizure discharges and this paroxysmal activity was not recorded in seven patients during clonazepam therapy. Two patients had progressive myoclonic epilepsy (Unverricht's Syndrome) and showed theta rhythms and spike wave activity in their EEG's; there was no change in their EEG's during clonazepam therapy. The other ten patients with myoclonic epilepsy showed no abnormalities. All patients with photosensitive epilepsy showed seizure activity with photo stimulation; clonazepam therapy suppressed this photosensitive response in all EEG's recorded during the trial. In all other patients EEG's were unchanged on clonazepam.

Effect of Clonazepam on Serum Concentrations of other Anticonvulsants

Phenytoin (Fig 6.2a) : The mean value of serum phenytoin in 16 patients was 49 $\mu\text{mol/l}$ (\pm 37 SD) before and 53 $\mu\text{mol/l}$ (\pm 42 SD) after 4 - 6 weeks on 3mg of clonazepam daily. The difference in the levels was not significant on a paired 't' test or a Wilcoxon signed-ranks test.

Phenobarbitone (Fig 6.2b) : The mean value of serum phenobarbitone in 14 patients was 56 $\mu\text{mol/l}$ (\pm 28 SD) before and 59 $\mu\text{mol/l}$ (\pm 31 SD) after 4 - 6 weeks on 3mg of clonazepam daily. This difference was not significant.

Primidone (Fig 6.2c) : In the ten patients taking this drug the mean serum level before treatment was 40 $\mu\text{mol/l}$ (\pm 28 SD). After clonazepam the mean was 42 $\mu\text{mol/l}$ (\pm 30 SD). There was no statistically significant difference.

Serum Concentrations of Clonazepam

Serum concentrations of clonazepam were measured in 19 patients in the double-blind trial and these are shown in Fig 6.3. A wide range of values was obtained and it was found that the patients with myoclonic epilepsy, who were mainly receiving only one drug other than clonazepam, and some of whom were on single therapy with clonazepam, showed higher levels than patients with temporal lobe epilepsy, who were receiving combined therapy with phenytoin and phenobarbitone or primidone. In particular, a significant negative correlation was found, in the sixteen patients receiving phenobarbitone or primidone, between clonazepam levels and phenobarbitone levels ($p < 0.01$) Fig 6.4. Since there was no effect of clonazepam on phenobarbitone levels, this would suggest that phenobarbitone has a depressive effect on clonazepam levels.

The initial response to clonazepam therapy in relation to serum levels of the drug is shown in Fig 6.5 for 19 patients in the double-blind trial and 8 patients in the

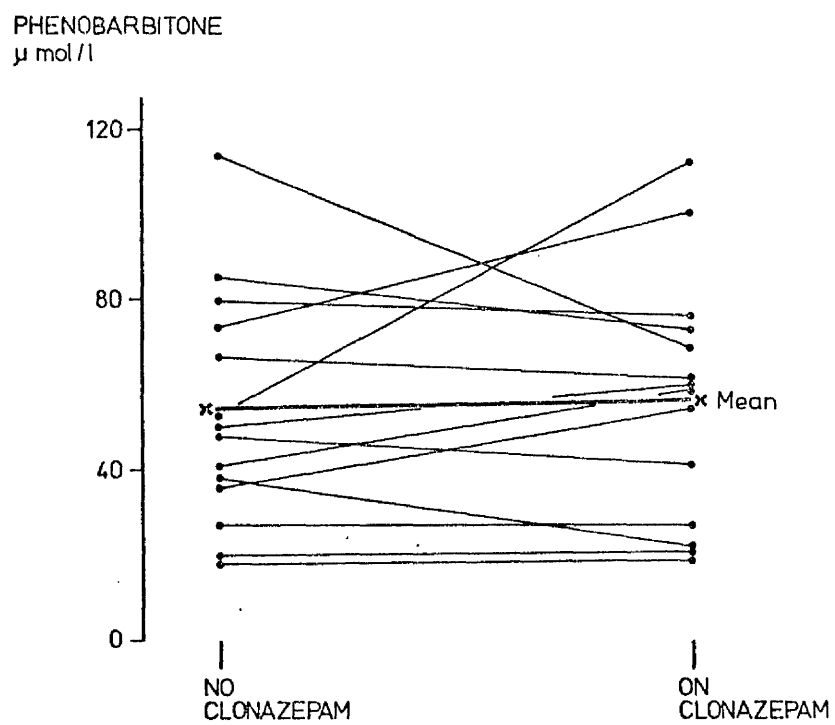


Fig 6.2b Effect of clonazepam therapy on
serum concentrations of phenobarbitone

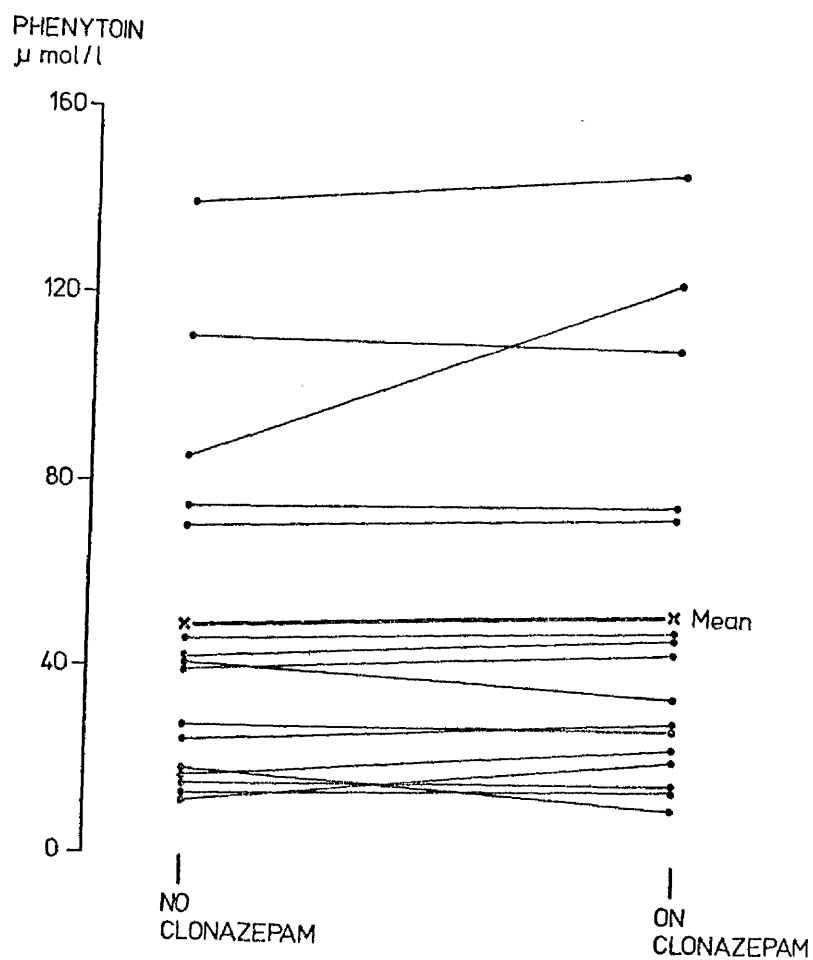


Fig 6.2a Effect of clonazepam therapy on
serum concentrations of phenytoin

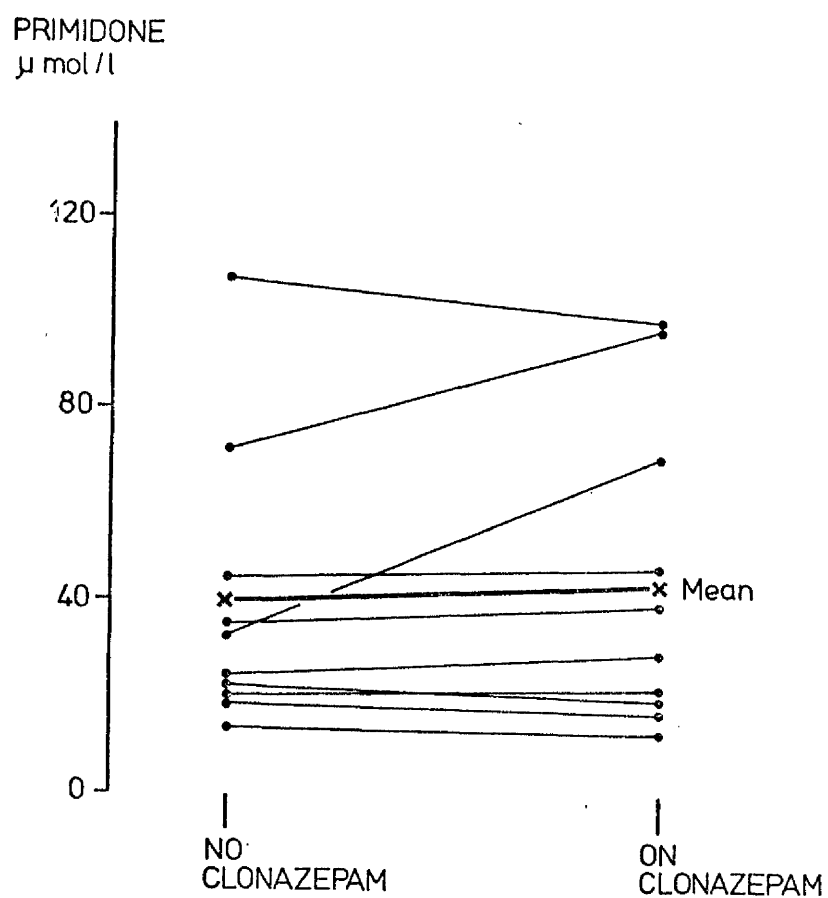


Fig 6.2c Effect of clonazepam therapy on serum concentrations of primidone

CLONAZEPAM
n mol/l

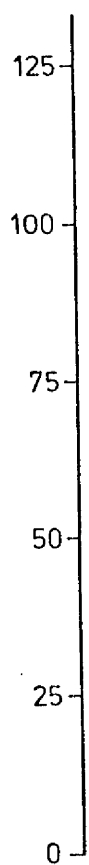


Fig 6.3

Serum clonazepam concentrations in
19 patients in the double-blind trial
who were receiving 3mg of the drug daily

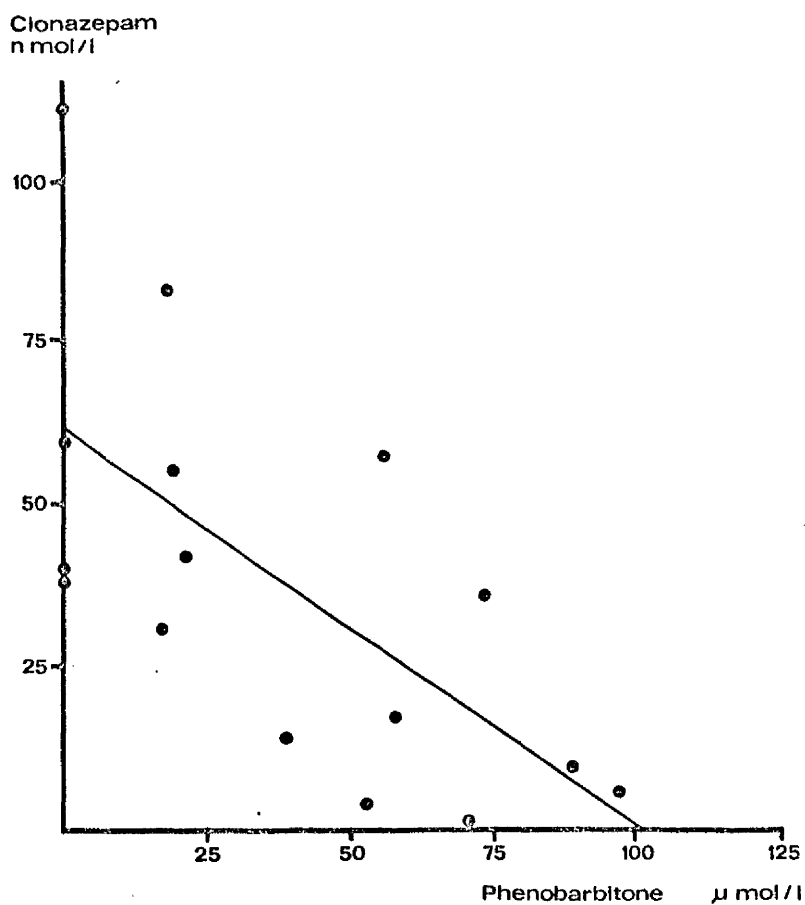


Fig 6.4

Relationship between serum clonazepam and phenobarbitone concentrations in 16 patients in the double-blind trial who were receiving, in addition to 3mg of clonazepam daily, phenobarbitone or primidone. A significant negative relationship ($p < 0.01$) was found

open trial who were receiving 3mg of clonazepam. It was found that patients showing complete abolition of seizures or a significant ($>50\%$) reduction in seizures tended to show serum levels higher than in those who showed little or no improvement. Patients showing abolition of seizures had a mean serum clonazepam concentration of 58 nmol/l (± 30 SD); those showing 50% reduction in seizures had a mean concentration of 49 nmol/l (± 15 SD); those showing no improvement had a mean concentration of 9 nmol/l (± 8 SD). Serum levels of clonazepam after one year of therapy, in 18 patients in whom dose was adjusted (2 - 12mg daily) to give maximum benefit, are shown in Fig 6.6; these were generally higher, with a mean of 116 nmol/l.

DISCUSSION

The results of these trials indicate that clonazepam is very effective in the treatment of myoclonic epilepsy. This is of considerable importance as this form of epilepsy was previously very difficult to treat (Van Woert and Sethy, 1975). The value of clonazepam in myoclonic epilepsy has previously been demonstrated only in uncontrolled studies (Barnett, 1973; Edwards and Eadie, 1973; Huang et al, 1973; Rett, 1973; Goldberg and Dorman, 1976). Clonazepam was also particularly effective in photosensitive epilepsy, which is in line with its potency in the Papio papio model of light-sensitive seizures (Killam et al, 1973). The drug was also found to be of value in patients with atypical absences and this has been found by other workers.

Results from both the double-blind trial and open trials suggest that clonazepam is of little value in the treatment of temporal lobe epilepsy (either for the major or the minor seizures), and there was only limited benefit in the small number of patients with generalised epilepsy with tonic-clonic seizures. This lack of benefit of clonazepam in the treatment of grand mal

CLONAZEPAM
n mol/l



Fig 6.6

Serum clonazepam concentrations
after one year of therapy in 18
patients in whom dose was adjusted
(2-12mg daily) to give maximum
benefit

seizures is in line with its low effectiveness in the prevention of maximal electroshock seizures in mice. Other clinical trials of the effectiveness of clonazepam in grand mal seizures have, however, yielded conflicting results. In ten uncontrolled studies with ten or more patients in whom clonazepam was added to the existing therapy, 10% to 70% (median 37%) had their grand mal attacks completely controlled and 10% to 96% (median 64%) had a 50% or greater reduction (Bergamini et al, 1970; Huang et al, 1973; Lehtovaara, 1973; Mikkelsen et al, 1973; Munthe-Kaas et al, 1973; Rosenmayr and Groh, 1973).

The total lack of effect of clonazepam in psychomotor seizures in patients in the present study with temporal lobe epilepsy is somewhat in conflict with findings by other workers. Seven large (17 or more patients) uncontrolled studies of clonazepam as an anticonvulsant adjunct have reported complete suppression of psychomotor in 26% to 57% (median 34%) of patients and a 50% or greater reduction in 35% to 89% (median 65%) (Boudin et al, 1972; Cass et al, 1973; Kick and Dreyer, 1973; Lehtovaara, 1973; Mikkelsen et al, 1973; Huang et al, 1974; Scollo-Lavizzari et al, 1974). It is possible that the daily dose of 3mg of clonazepam in the trials reported here was insufficient for control, as a higher dosage was reported as being necessary in temporal lobe epilepsy (Huang et al, 1973). There appeared to be a greater action of clonazepam against psychomotor seizures when 6mg of the drug was used to treat patients in a double-blind trial against sodium valproate and this is described in Chapter 7.

Conflicting reports have previously suggested that clonazepam may either raise or lower phenytoin or phenobarbitone serum concentrations (Edwards and Eadie, 1973; Daurella, 1974; Hara et al, 1976), but it has also been concluded (Huang et al, 1973) that there is no constant effect of clonazepam medication upon serum concentrations of other anticonvulsants and that an

increase in phenytoin concentrations might be due to more constant drug intake because of closer supervision. The observations in the present study indicate that serum concentrations of phenytoin, phenobarbitone and primidone are not altered by the addition of clonazepam. It is unlikely that any of the therapeutic or toxic effects of clonazepam administration were due to alteration in the serum levels of other drugs.

Administration of other drugs did appear to have an effect on serum levels of clonazepam. The results suggest that treatment with other anticonvulsants, particularly phenobarbitone or primidone, lowers serum concentrations of clonazepam presumably via enzyme induction. This may be of therapeutic importance since the patients in the double-blind trial with temporal lobe and tonic-clonic epilepsy, who responded poorly to clonazepam therapy, were being treated with high doses of other anticonvulsants and showed high serum concentrations of phenobarbitone and low concentrations of clonazepam. It has previously been reported (Naestoft et al, 1973; Dreifuss et al, 1975) that, after daily oral doses of 1.5mg - 4mg in patients taking clonazepam alone, serum concentrations ranged from 40 - 240 nmol/l. It was found that patients in the double-blind trial who showed clonazepam concentrations below 40 nmol/l were those who responded poorly to the drug.

Several reports of clonazepam therapy have mentioned the relatively high frequency of side-effects as a major problem (Edwards and Eadie, 1973; Huang et al, 1973; Lund, 1973), although these may disappear as treatment is continued. In the present study, as in others, the most frequent side-effects were various degrees of drowsiness and ataxia which necessitated dose reduction and eventual discontinuation in some patients. In general, side-effects appear to be dose-related since there was more of a problem in patients on multiple anticonvulsant therapy receiving 6mg clonazepam daily (see Chapter 7) than in the patients receiving 3mg daily. However, patients receiving lower or no doses of other

anticonvulsants were able to tolerate doses of up to 12mg without problems.

There appeared to be a negative correlation between serum levels of clonazepam and side-effects, since patients on high doses of other anticonvulsants showed reduced serum levels but increased side-effects. Previous studies have shown that side-effects can be reduced by reducing doses of phenytoin (Huang et al, 1973) or barbiturates (Edwards and Eadie, 1973). The present study shows that the side-effects are not due to an increase in serum levels of phenytoin or phenobarbitone with clonazepam therapy, but it is possible that there is a combined sedative effect of the drugs. However, an alternative hypothesis is that clonazepam is converted to a toxic metabolite in the presence of enzyme-inducing drugs. There is no correlation between side-effects of clonazepam and its major metabolite, 7-amino-clonazepam, but treatment with phenytoin lowers levels of this metabolite (Sjö et al, 1973) and it is likely that enzyme induction causes the involvement of some other metabolite pathway.

SUMMARY

1. A double-blind and an open trial have been conducted to assess the value of clonazepam therapy in patients with epilepsy. Clonazepam was found to be particularly effective in myoclonic and photosensitive epilepsy.
2. Clonazepam administration did not cause any change in serum levels of phenytoin, phenobarbitone or primidone.
3. Serum levels of clonazepam were lower in patients receiving large doses of other anticonvulsant drugs and there was a negative correlation between serum levels of clonazepam and serum levels of phenobarbitone. The patients showing low levels of clonazepam were those who showed no improvement in seizure control on the drug.

4. Side -effects of clonazepam therapy were greater in patients who were receiving high doses of other anticonvulsant drugs.

CHAPTER 7

HOW DOES SODIUM VALPROATE COMPARE WITH CLONAZEPAM AS AN ANTICONVULSANT?

INTRODUCTION

Chapter 6 described trials to assess the value of the new benzodiazepine anticonvulsant clonazepam. Sodium valproate (Epilim^R) is another new anticonvulsant currently under evaluation. Since its introduction in 1963 the drug has been widely used as an anti-epileptic drug in European continental countries, but has only recently been introduced into Britain and has not yet been introduced into America.

Whereas most of the currently available anticonvulsant drugs have chemical features in common, sodium valproate is a different entity with a simple chemical structure (Fig 7.1) which (unlike other drugs) does not contain nitrogen. It has good anticonvulsant activity in audiogenic seizures in mice (Simler et al, 1968) and in seizures induced electrically or by CO₂ withdrawal (Swinyard, 1964). A study in rats (Godin, 1968) suggested that the anticonvulsant effect of sodium valproate is related to an increase in the level of γ -aminobutyric acid (GABA), by an inhibition of GABA-transaminase.

The introduction of new anticonvulsants requires their assessment both individually and comparatively. Although sodium valproate and clonazepam have been tested independently in a number of trials, comparative assessment of their value is desirable. One comparative trial has been reported (Lance and Anthony, 1977) but it was carried out as an open trial and there was no study of effects on serum levels of other drugs. This Chapter describes results of a controlled crossover trial of sodium valproate and clonazepam in the same patients, all of whom were initially poorly controlled. In addition,

serum levels of other anticonvulsants were monitored in order to distinguish the direct effects of these drugs from the secondary elevation of other anticonvulsants. In particular, sodium valproate has been reported to increase phenobarbitone serum concentrations (Richens and Ahmad, 1975; Schobben, 1975; Vakil et al, 197), and it was attempted to keep phenobarbitone concentrations relatively constant by reducing the dose when necessary.

In Chapter 6 it was found that clonazepam in a dose of 3mg daily was ineffective in patients with temporal lobe epilepsy. In the comparative trial a higher dose of 6mg was given to determine whether this produced a better response.

PATIENTS AND METHODS

Thirty-two adult epileptic patients who were resident in Quarrier's Homes were studied in the double-blind trial. There were 15 males and 17 females aged 21 - 63 years (mean 36 years). The drugs which they were receiving were phenytoin (20 patients), phenobarbitone (17 patients), primidone (14 patients), carbamazepine (10 patients) ethotoin (2 patients) and pheneturide (1 patient). The criteria of selection was that they were having more than five seizures per month for the preceding three months and that two estimations of anticonvulsant serum levels should have been carried out in that period, the values being within the therapeutic ranges for phenytoin ($40 - 80 \mu\text{mol/l}$) or phenobarbitone ($> 40 \mu\text{mol/l}$). Twenty-nine patients had frequent major seizures (generalised tonic-clonic fits) and the patients all suffered from minor seizures (absences or focal attacks). The patients were classified on the basis of the EEG abnormalities into generalised or focal epilepsy.

The trial consisted of three consecutive periods each of twelve weeks and the design is shown in Fig 7.2. Initially the patients were observed for twelve weeks during which serum concentrations of anticonvulsants were measured twice. The 32 patients were then randomly divided into

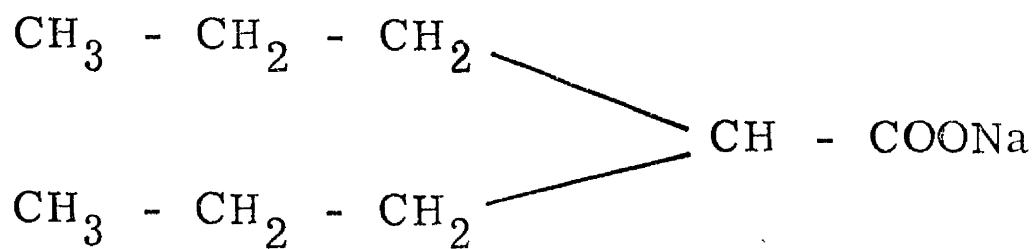


Fig 7.1 Chemical structure of sodium valproate

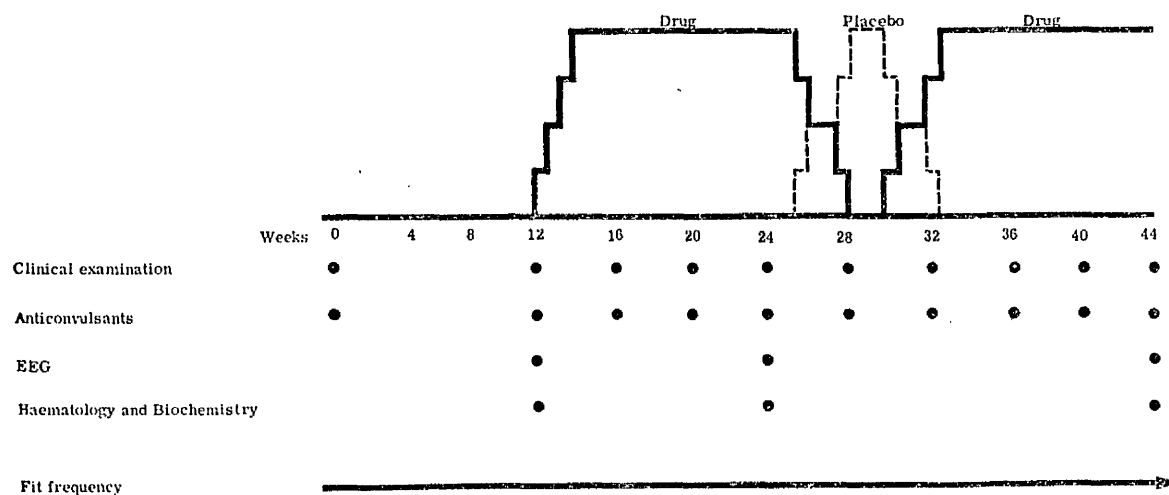


Fig 7.2 Design of cross-over trial of sodium valproate and clonazepam

two groups, each of 16 patients. One received sodium valproate (400mg four times daily) and the other received clonazepam (2mg three times daily). In some patients the dose of clonazepam was later reduced because of side-effects. After 12 weeks each group crossed over to the other drug over 6 weeks using a placebo matching the first additional drug they received. Serum concentrations of anticonvulsants were measured at 4 week intervals. The seizures were recorded by the nursing staff as major and minor fits.

Serum concentrations of phenytoin, phenobarbitone, primidone and PEMA were measured by gas chromatography and clonazepam was measured by radioimmunoassay as before. In addition, sodium valproate was measured by a gas chromatographic technique (see Appendix).

RESULTS

Seizure Frequency

Fifteen patients completed all parts of the trial. The remaining seventeen patients completed one part of the trial only, twelve completing the sodium valproate treatment period and five the clonazepam treatment period only. Eight of these patients left the trial after the first drug period for various reasons unrelated to drug therapy. Another nine patients stoppped taking the trial drug because of side-effects; eight were on clonazepam and one on sodium valproate.

When the average number of seizures per month before and after treatment are compared for the fifteen patients who completed the trial, sodium valproate ($p < 0.005$, Wilcoxon signed-ranks test) and clonazepam ($p < 0.02$) both reduced the frequency of minor seizures but with sodium valproate having the greater effect (Fig 7.3). Major seizures were also reduced with sodium valproate but this failed to reach significance. The effects of treatment are summarised according to the percentage improvement for

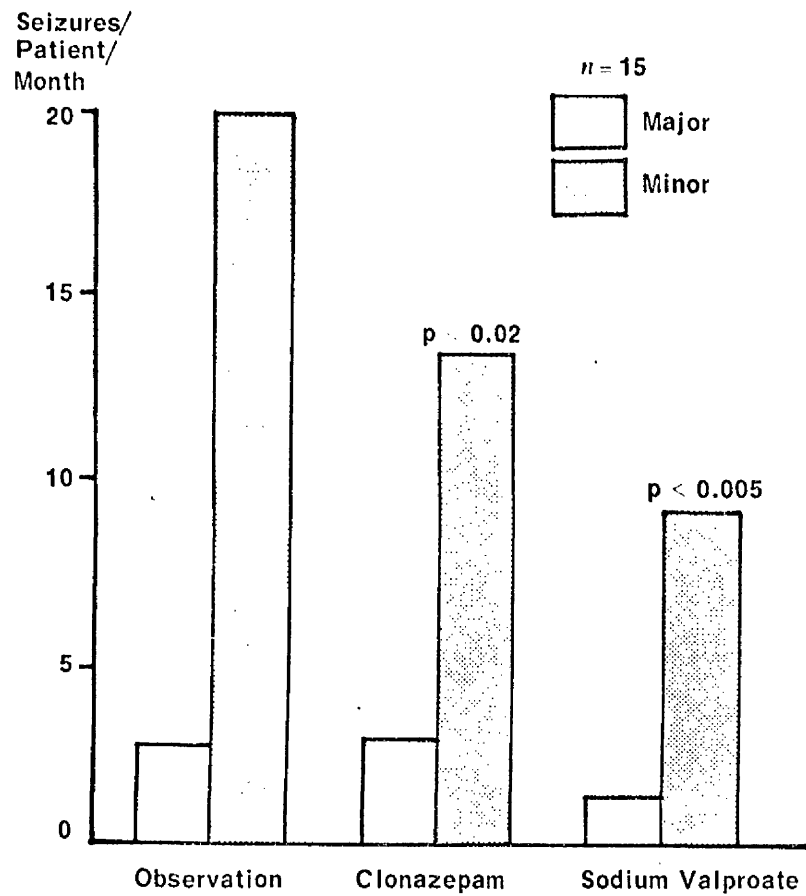


Fig 7.3 Effects of clonazepam and sodium valproate on mean frequencies of major and minor seizures in 15 patients who completed comparative trail

TYPE OF EPILEPSY	SODIUM VALPROATE						CLONAZEPAM					
	No	100%	75-100%	50-75%	50%	Worse	No	100%	75-100%	50-75%	50%	Worse
MINOR SEIZURES												
Generalised	13	1	-	7	4	1	9	-	-	3	4	2
Temporal Lobe	8	-	1	1	5	1	7	-	-	-	3	4
Focal Motor	4	-	1	1	1	1	3	-	1	-	1	1
Unclassified	2	1	-	-	1	-	1	-	1	-	-	-
TOTAL	27	2	2	9	11	3	20	-	2	3	8	7
MAJOR SEIZURES												
Generalised	12	2	2	3	2	3	8	-	-	1	5	2
Temporal Lobe	7	3	-	2	-	2	7	-	1	1	1	4
Focal Motor	3	-	2	-	-	1	2	-	-	-	-	2
Unclassified	2	1	-	-	1	-	1	1	-	-	-	-
TOTAL	24	6	4	5	3	6	18	1	1	2	6	8

Table 7.1

The effect of treatment with clonazepam and sodium valproate on the frequency of major and minor seizures in all the patients who were treated with one or both drugs

CLONAZEPAM											
SODIUM VALPROATE											
TYPE OF EPILEPSY	No	100%	75-100%	50-75%	50%	Worse	100%	75-100%	50-75%	50%	Worse
MINOR SEIZURES											
Generalised	8	1	-	6	1	-	-	-	2	4	2
Temporal Lobe	5	-	-	-	4	1	-	-	-	2	3
Focal Motor	1	-	-	1	-	-	-	1	-	-	-
Unclassified	1	1	-	-	-	-	-	1	-	-	-
TOTAL	15	2	-	7	5	1	-	2	2	6	5
MAJOR SEIZURES											
Generalised	7	1	1	2	2	1	-	-	1	4	2
Temporal Lobe	5	2	-	1	-	2	-	-	1	1	3
Focal Motor	-	-	-	-	-	-	-	-	-	-	-
Unclassified	1	1	-	-	-	-	1	-	-	-	-
TOTAL	13	4	1	3	2	3	1	-	2	5	5

Table 7.2 The effect of treatment with clonazepam and sodium valproate on the frequency of major and minor seizures in the 15 patients who completed the trial

all the patients treated in Table 7.1 and for the 15 patients who completed the trial in Table 7.2.

Side Effects

The main side-effects with sodium valproate were drowsiness (25% of patients) and ataxia (17% of patients). These were abolished in all but one patient by reducing the dose of phenobarbitone or primidone. In one patient the drug was stopped because of drowsiness.

Side-effects were more of a problem with clonazepam, with 65% of patients showing drowsiness and 55% of patients showing ataxic symptoms. Because of side-effects it was necessary to reduce the clonazepam dose in 17 of the 29 patients in whom treatment was started from 6mg/day to 1 - 5 (mean 2.9) mg/day and the drug was eventually stopped in eight of these patients.

Serum Concentrations of Sodium Valproate

The mean serum concentrations of sodium valproate for patients on the trial, all receiving 1600mg daily, was $497 \pm 184\text{SD } \mu\text{mol/l}$. The variation was generally reasonably small, with 21 of the 26 patients (blood samples were not obtained from one patient) showing concentrations within the range 350 - 700 $\mu\text{mol/l}$. The variation in serum concentrations could not be explained in terms of variation in weight of the patients ; no correlation was found between serum concentrations and weight, which ranged from 47 - 76Kg (mean 62Kg). There was no apparent relationship between serum concentrations and therapeutic effect : 18 patients who showed a significant (>50%) reduction in the total number of seizures had a mean serum concentration of 490 $\mu\text{mol/l}$ and 8 patients who showed no improvement had a mean concentration of 513 $\mu\text{mol/l}$.

Serum Concentrations of Clonazepam

The serum concentrations of clonazepam showed rather more variation than sodium valproate. The mean serum

concentration for 23 patients in whom blood samples were obtained while they were receiving 6mg daily was 82.3 ± 37.8 SD nmol/l, but a number of patients actually showed lower serum concentrations of clonazepam throughout the major part of the trial period since doses were reduced. Twelve patients on doses ranging from 1 - 5mg daily (mean 2.9mg) showed a mean level of 29.1 ± 19.1 SD nmol/l. There appeared to be some relationship between serum concentrations and the therapeutic effect of clonazepam. The serum concentrations for the 20 patients treated are shown in Fig 7.4, for patients showing significant improvement (>50% reduction in seizures) and patients showing no improvement. For patients in whom the initial dose was reduced during the first month the improvement in seizure control has been assessed during the second two months only and it is the serum concentration obtained on the lower dose that is given.

Although patients generally responded better to sodium valproate, some of the patients in whom high serum concentrations of clonazepam were obtained did show considerable improvement on this drug. Fig 7.5 shows the effect of clonazepam on minor seizures in an individual patient - the seizure frequency is shown not only for a three month period before the trial but for the entire period during which the drug regime was unchanged. This patient was receiving only 3mg of clonazepam after the first two weeks of the trial but showed a relatively high serum concentration (67.2 nmol/l) on this dose. It is notable that, although this patient was on reasonably high doses of anticonvulsant drugs (200mg phenytoin, 120mg phenobarbitone) there was no enzyme-inducing effect as measured by raised serum concentrations of (γ GT 17U/l) - the possible significance of this is discussed in Chapter 12.

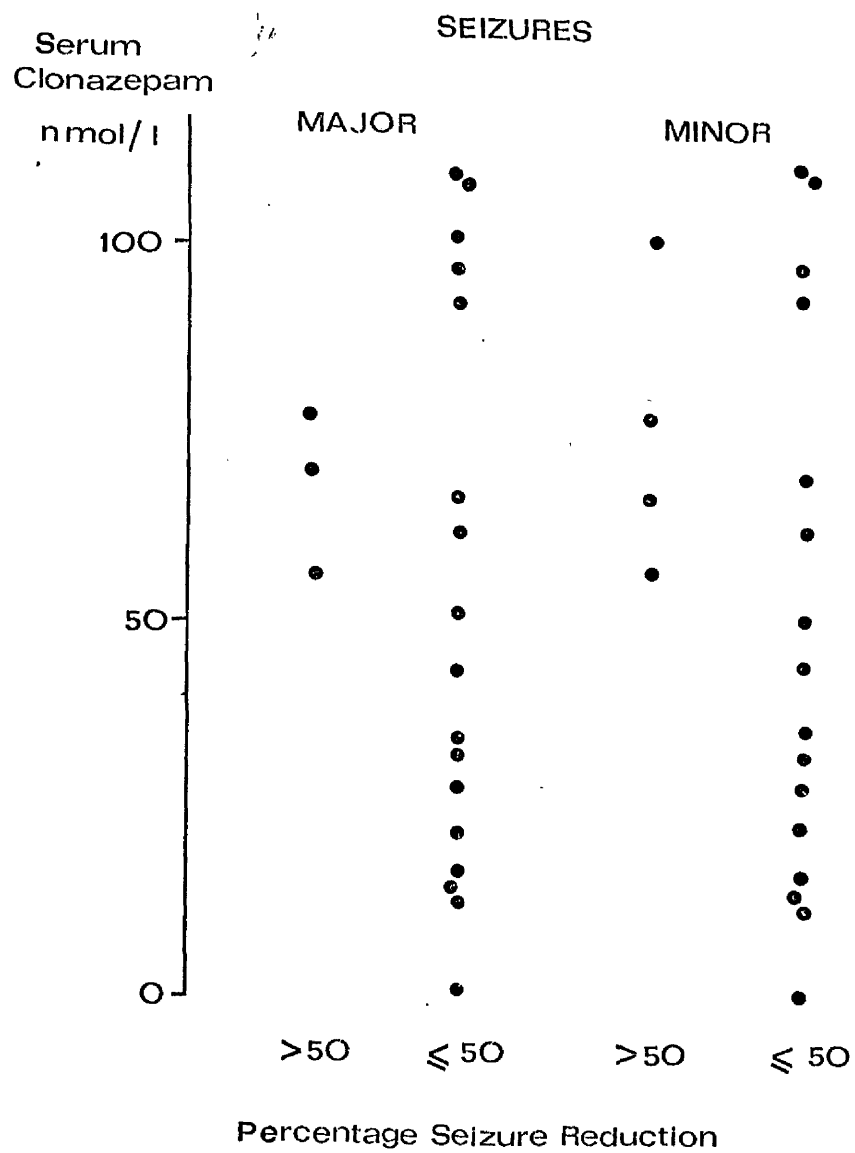


Fig 7.4 Serum concentrations of clonazepam and therapeutic effect for 20 patients in comparative trial

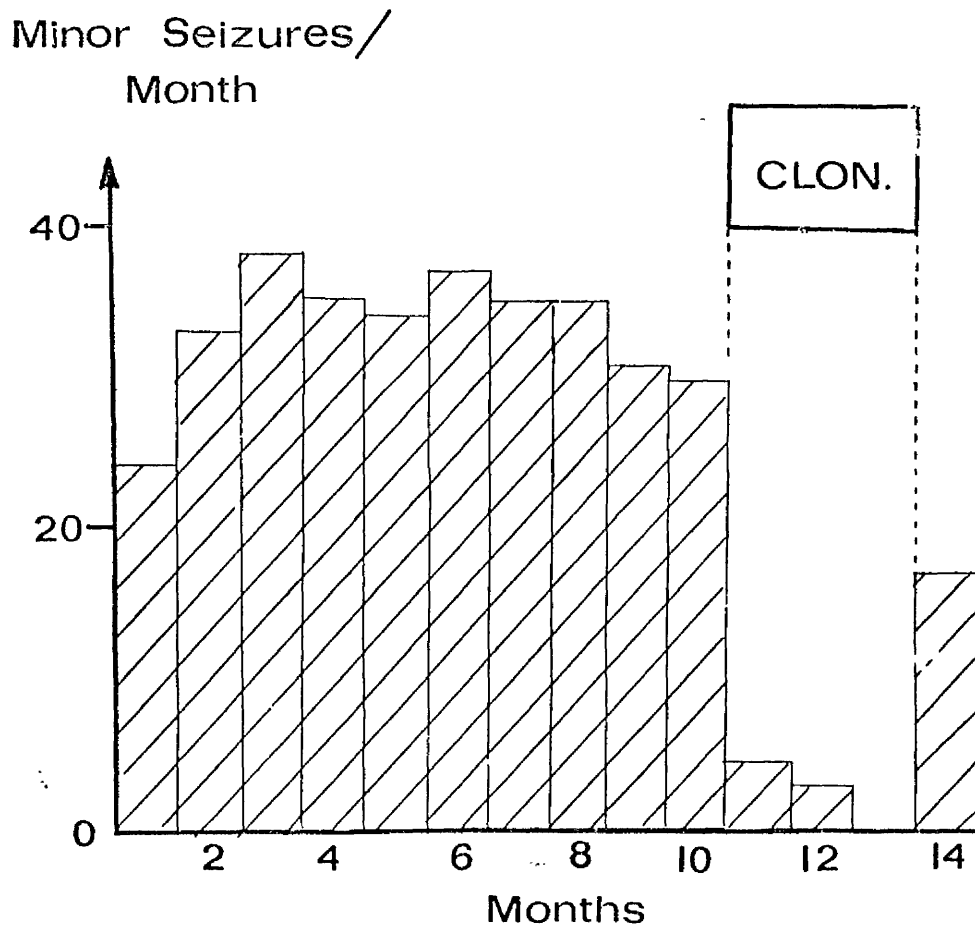


Fig 7.5 Effect of clonazepam therapy on
frequency of minor seizures in
an individual patient

Serum Concentrations of Other Drugs

Doses of other anticonvulsants were reduced during the trial if, on administration of the trial drug, there was an increase of 15% in serum concentration. Dose reduction was necessary during sodium valproate treatment in seven patients who showed an increase in phenobarbitone serum concentrations (five were receiving phenobarbitone and two were receiving primidone) and one patient also showed an increase in phenytoin serum concentration. There were no significant effects of clonazepam treatment on serum concentrations of other drugs.

Overall, on first administration of sodium valproate there was a significant increase in phenobarbitone concentrations in patients receiving phenobarbitone ($p < 0.05$, Wilcoxon signed-ranks test) but there was no effect in patients receiving primidone (Fig 7.6a, b). Two patients were receiving phenobarbitone plus primidone - these both showed a 10% rise in serum concentrations of phenobarbitone.

In patients receiving primidone, there was also no noticeable effect of sodium valproate on serum concentrations of primidone nor, in the three patients in whom this metabolite was measured, PEMA.

In 14 patients in whom serum phenytoin concentrations were measured after sodium valproate administration (blood samples were not obtained from one patient), there was a significant fall ($p < 0.05$) in concentrations (Fig 7.6c). This effect was generally most marked in patients in whom the phenobarbitone serum concentrations did not rise; indeed, in one patient in whom the phenobarbitone concentration increased the phenytoin concentration also increased and it was necessary to reduce the phenytoin dose.

No significant effects on serum concentrations of other anticonvulsants were observed with clonazepam therapy.

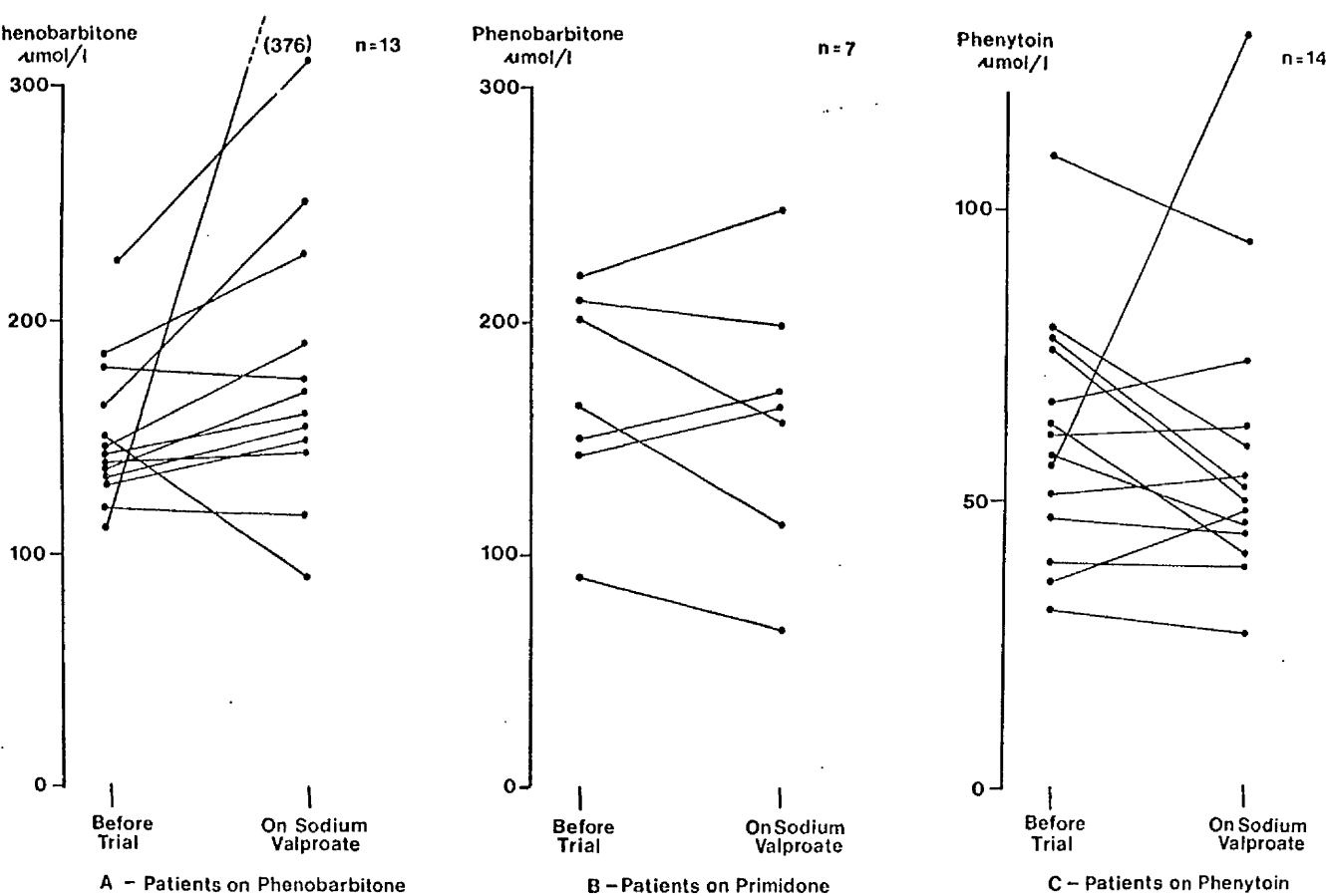


Fig 7.6a,b,c Effects of sodium valproate therapy on serum concentrations of phenobarbitone (patients on phenobarbitone and patients on primidone) and of phenytoin . There was a significant rise ($p < 0.05$) in serum phenobarbitone concentrations in 13 patients receiving phenobarbitone. There was a significant fall ($p < 0.05$) in serum phenytoin concentrations after sodium valproate administration

DISCUSSION

Both sodium valproate and clonazepam significantly reduced the frequency of minor seizures when added to the existing therapy of 27 patients whose fits had proved difficult to control. Major seizures were also reduced by sodium valproate, but this did not reach a level of significance. Clonazepam was generally less effective than sodium valproate although it appeared to be of value in a few patients. The lack of effect of clonazepam in patients on multiple drug therapy, particularly on psychomotor seizures in patients with temporal lobe epilepsy, corresponds with results found in other trials using a lower dose which are discussed in the previous Chapter.

Sodium valproate appeared to be more effective in reducing seizures in patients whose EEGs showed generalised abnormalities than in those with focal abnormalities, and this accords with results in other studies. A number of open trials, particularly in children, have reported sodium valproate to be less effective in focal epilepsy and especially psychomotor seizures (Kerfriden et al, 1970; Völzke and Dose, 1973; Jeavons and Clark, 1974; Barnes and Bower, 1975), although other investigators have reported the drug to be equally effective in temporal lobe epilepsy (Beaumanoir, 1973; Salzarula and Lairy, 1974; Espir et al, 1976; Grant and Barot, 1976; Hassan et al, 1976). Sodium valproate was more effective than clonazepam in this study, both in the treatment of generalised and of temporal lobe epilepsy. This is in conflict with findings in another comparative trial (Lance and Anthony, 1977), in which clonazepam was more effective than sodium valproate in temporal lobe epilepsy. However, this was an uncontrolled trial and the drugs were not in most instances compared in the same patients. Also, in 70% of patients the most recently added drug was withdrawn as clonazepam was added and this may have produced an overall lower level of medication than in the patients in this study, which might have increased the effectiveness of clonazepam (see Chapter 6).

These investigators used lower doses of sodium valproate than in the present trial.

Variations in response to sodium valproate therapy between different patients did not appear to be related to variations in serum concentrations of the drug. The serum concentrations of sodium valproate are fairly highly correlated with the daily dose of the drug (see Chapter 3) and there was a fairly small variation in serum concentrations in patients in the trial who were all receiving the same dose. Most patients in the trial showed serum levels of sodium valproate in the range 350 - 700 $\mu\text{mol/l}$, and other workers (Meinardi et al, 1974; Chard et al, 1976; Espir et al, 1976; Grant and Barot, 1976) have found that therapeutic levels lie in this range. There was no apparent effect of administration of other anticonvulsant drugs on serum concentrations of sodium valproate. By comparison, serum concentrations of clonazepam showed wide variation between different individuals and the therapeutic response to the drug appeared to be related to the serum concentrations. Administration of other anticonvulsant drugs appears to lower serum concentrations of clonazepam and this effect is discussed in Chapters 6 and 12.

Administration of other anticonvulsants did not affect serum concentrations of sodium valproate, but sodium valproate affects serum concentrations of phenobarbitone and possibly also of phenytoin. In this trial doses of phenobarbitone or primidone were reduced in patients who showed an increase in serum concentrations of phenobarbitone of more than 15% on administration of sodium valproate. This occurred in 7 patients, of whom five were receiving phenobarbitone and two were receiving primidone. Six of these patients showed drowsiness on first administration of sodium valproate which was abolished by reducing the serum concentration of phenobarbitone or primidone. The mechanism of the effect of sodium valproate on phenobarbitone levels is unknown, but the most likely hypothesis is that sodium valproate, being a fatty acid and therefore binding strongly to hepatic microsomes, blocks the metabolism of phenobarbitone in the liver.

There was a significant overall increase in serum concentrations of phenobarbitone in patients receiving phenobarbitone itself but not in patients receiving primidone. This suggests the possibility that sodium valproate may inhibit the metabolism of primidone to PEMA so that less of the drug is converted to phenobarbitone. No influence of sodium valproate was found on the PEMA : primidone ratio in serum and this might suggest that sodium valproate inhibits primidone metabolism to phenobarbitone. No effect of sodium valproate on primidone serum concentrations was found, but since only about 25% of the drug is metabolised to phenobarbitone, an inhibition of this metabolism would produce a larger increase in phenobarbitone serum concentrations than it would a decrease in primidone serum concentrations.

Since the phenobarbitone or primidone dose was reduced in patients in the trial in whom serum concentrations of phenobarbitone showed a significant increase, it is likely that most of the therapeutic benefit obtained with sodium valproate therapy was due to the action of the drug itself. However, evaluation of the drug in individual patients should take the possibility of drug interactions into account. For example, Fig 7.7 shows serum drug concentrations and seizure control for a patient in whom the phenobarbitone serum concentration showed a dramatic increase on sodium valproate administration ; the period before the phenobarbitone dose was reduced showed the lowest seizure frequency.

Conflicting reports have previously suggested that administration of sodium valproate may reduce (Penry et al, 1976; Vakil et al, 1976) or increase (Windorfer et al, 1975; Vajda et al, 1976) serum concentrations of phenytoin, although these effects may be temporary. It has been suggested that sodium valproate may displace phenytoin from its plasma protein binding sites (Patsalos and Lascalles, 1977). A significant decrease in phenytoin concentrations was found in the present study, particularly for the patients in whom phenobarbitone serum concentrations did not increase - of nine patients receiving

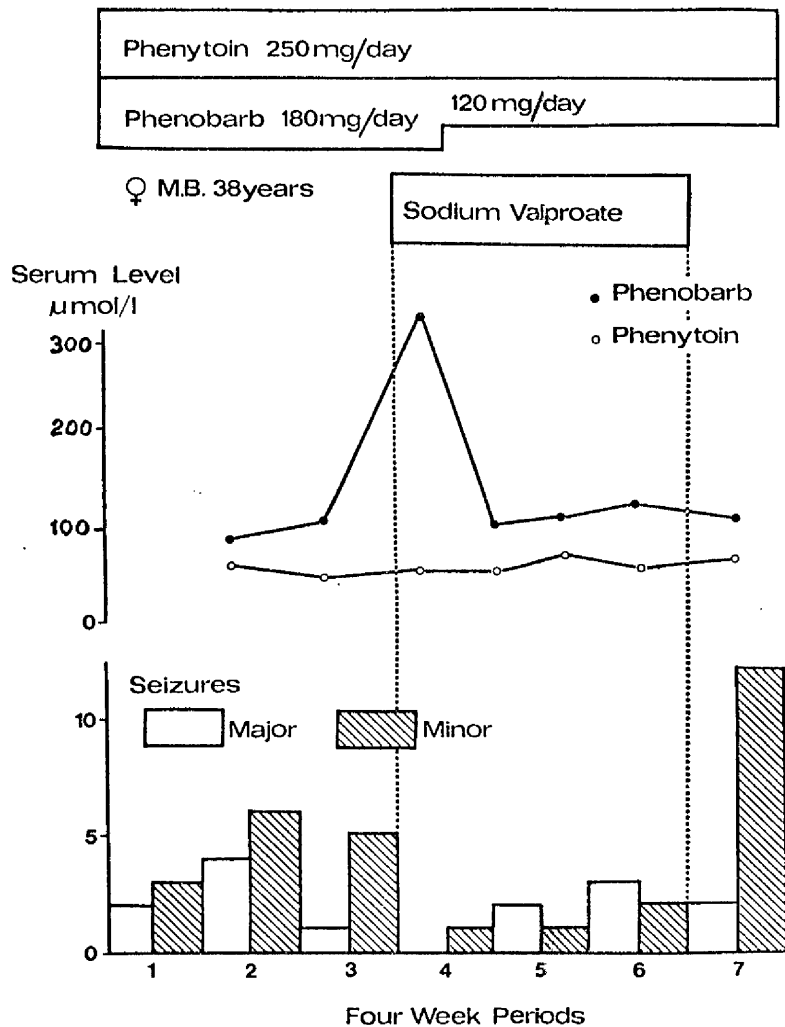


Fig 7.7

Effects of sodium valproate therapy on seizure frequency and serum concentrations of phenytoin in an individual patient. This particular patient showed a large increase in serum phenobarbitone concentration on first administration of sodium valproate, accompanied by toxic symptoms. These toxic symptoms disappeared when the phenobarbitone dose was reduced

phenytoin the phenytoin serum concentration was reduced in eight and unchanged in one. However, in five patients receiving phenytoin in whom phenobarbitone serum concentrations were raised by more than 15% the serum concentration was reduced in one, unchanged in two and raised in two; one of these patients indeed showed a 100% increase in phenytoin concentration and toxic effects which required dose reduction. It is possible that in these patients the raised phenobarbitone concentrations may cause inhibition of phenytoin metabolism (as described in Chapter 4) and this counteracts the action of sodium valproate.

In conclusion, sodium valproate is generally a more useful anticonvulsant than clonazepam when given to patients on multiple drug therapy. However, it is important to monitor serum concentrations of other anticonvulsants during treatment with sodium valproate as these may influence seizure control or cause side effects. It is possible that some of the therapeutic effect of sodium valproate in patients in this trial may have been due to a displacement of phenytoin from its plasma protein binding sites ; the clinical importance of variations in protein binding of drugs is discussed in the following section.

SUMMARY

1. A cross-over trial has been conducted to compare the effectiveness of clonazepam and sodium valproate therapy in epileptic patients already receiving other anticonvulsant drugs. Both drugs produced a significant reduction (sodium valproate $p < 0.005$, clonazepam $p < 0.02$) in minor seizures. with sodium valproate having the greater effect.
2. Administration of other anticonvulsants did not affect serum concentrations of sodium valproate but sodium valproate caused a significant rise in phenobarbitone concentrations in several patients.

The phenobarbitone dose was reduced in patients in whom phenobarbitone serum concentrations showed an increase of more than 15%. With sodium valproate treatment, phenobarbitone concentrations increased significantly ($p < 0.05$) in patients receiving phenobarbitone but not in patients receiving primidone. Phenytoin concentrations were reduced ($p < 0.05$) during treatment with sodium valproate.

3. Side-effects were a problem in patients receiving clonazepam and the dose of the drug had to be reduced or even stopped in many patients. This may have lowered the value of clonazepam therapy, since the effectiveness of the drug appeared to be related to serum levels.
4. It is concluded that sodium valproate is generally a more valuable drug than clonazepam in patients on multiple drug therapy.

CHAPTER 8

PROTEIN BINDING OF PHENYTOIN AND PHENOBARBITONE

INTRODUCTION

Most drugs are carried from their sites of absorption to their sites of action by the circulating blood. Some drugs are simply dissolved in serum water, but many others are partly associated with plasma proteins, particularly albumin. Only free drug can reach the site of action and exert a pharmacologic effect and it would be expected that the action of a drug would depend on the degree of protein binding, although there are a few studies of this relationship in man (Kunin, 1966; Borg^o et al, 1970).

Chapters 3 - 7 have shown that determination of anticonvulsant serum concentrations is of value in the management of epilepsy, but it was the total serum concentration which was measured. If there is much variation among patients in the degree of anticonvulsant protein binding, and if the binding determines brain concentration, it is likely that the free concentration will correlate more accurately than the total concentration with clinical effectiveness. There have been a considerable number of studies of phenytoin binding in man (Table 8.1) which have shown that phenytoin is highly bound to plasma proteins and the binding may be affected by many factors including protein levels (Porter and Layzer, 1975), disease states (Reidenberg et al, 1971; Hooper et al, 1974; Blasche et al, 1975; Olsen et al, 1975) and the presence of other drugs (Lunde et al, 1970).

In the previous Chapter it was shown that sodium valproate administration reduced serum concentrations of phenytoin and it was suggested that this may be due to displacement of phenytoin from plasma proteins.

YEAR	AUTHOR	% BOUND	METHOD	TEMPERATURE
1960	Svensmark et al	75	CSF/Plasma	-
1960	Triedman et al	88	CSF/Plasma	-
1963	Firemark et al	80	Ultrafiltration	37C
1969	Viukari and Tammisto	45	CSF/Plasma	-
1970	Lunde et al	94	Ultrafiltration	Room Temp
	Lunde et al	91	Equilibrium dialysis	Room Temp
1971	Reidenberg	93	Ultrafiltration	Room Temp
1971	Ehrnebo et al	89	Equilibrium dialysis	37C
1971	Conard et al	96	Equilibrium dialysis	4C
1972	Blum et al	90	Ultrafiltration	37C
1972	Reynolds et al	85	CSF/Plasma	-
1972	Lund et al	90	CSF/Plasma	-
	Lund et al	90	Ultrafiltration	37C
1973	Booker and Darcey	91	Ultrafiltration Cones	22C
1974	Vajda et al	88	CSF/Plasma	-
1974	Hooper et al	89	Ultrafiltration	37C
1974	Kurata and Wilkinson	95	Ultrafiltration	Room Temp
	Kurata and Wilkinson	91	Equilibrium dialysis	Room Temp
1975	Blaschke et al	90	Ultrafiltration	37C
1975	Porter and Layzer	91	Ultrafiltration	25C
1975	Olsen et al	94	Ultrafiltration	Room Temp
1976	Sherwin et al	89	Equilibrium dialysis	Room Temp
1976	Odar-Caderlöf and Borga	87	Equilibrium dialysis	37C

Table 8.1 Summary of Previous Data on Phenytoin Binding
in Normal Adult Man

This Chapter describes a study of plasma protein binding of phenytoin and phenobarbitone in epileptic patients receiving these drugs. Values obtained for binding obtained by different techniques have been compared and the degree of variation in binding of these drugs between different individuals have been assessed. In addition, in vitro studies have been carried out to examine the effects of temperature, plasma dilution and sodium valproate administration on protein binding. The degree of binding of phenytoin to albumin relative to other proteins has been measured by separation of the protein fractions on Sephadex and the importance of variations in albumin concentrations between different patients is shown.

PATIENTS AND METHODS

CSF Concentrations of Anticonvulsants

CSF was obtained from 39 epileptic inpatients on phenytoin therapy. Phenytoin concentrations in the CSF and in serum were measured by a radioimmunoassay technique (Paxton et al, 1976; see Appendix) requiring only small volumes of fluid. In some patients sufficient CSF was obtained for measurement of anticonvulsant concentrations by GLC, and phenobarbitone (17 patients) and primidone (13 patients) concentrations were measured and compared with serum concentrations.

Protein Binding of Phenvtoin and Phenobarbitone

Protein binding was measured both by ultrafiltration using the method of Lunde et al (1970) and by equilibrium dialysis. The plasma was labelled with radioactive phenytoin and phenobarbitone and drug concentrations in the ultrafiltrate or dialysate were determined by scintillation counting. In addition, the effect of sodium valproate in vitro on protein binding of phenytoin and phenobarbitone was measured by ultrafiltration but using an Amicon ultrafiltration cell rather than filtration through cellulose tubing. Details of all these methods are given in the Appendix.

Protein binding in serum from 63 epileptic patients was determined by ultrafiltration at room temperature. In 28

of these patients, serum albumin concentrations were determined.

In vitro studies were performed using the equilibrium dialysis technique, with plasma obtained from a blood bank. The effects of temperature and plasma dilution (diluting plasma with isotonic phosphate buffer) were determined.

Binding of Phenytoin to Plasma Proteins Separated on Sephadex G100

1ml of human plasma was equilibrated for 1 hour at room temperature with 20 μg ^{14}C -labelled phenytoin (Radiochemical Centre, Amersham). Sephadex G-100 (Pharmacia Fine Chemicals Ltd) pre-equilibrated with 0.1M phosphate buffer (pH 7.2) was packed in a column (30cm long x 1cm diameter) and 0.5ml of labelled plasma was applied to the column. The protein was eluted with phosphate buffer (flow rate 0.5ml/min) and the eluant collected in 0.5ml fractions.

0.2ml of each fraction was diluted with 2.8ml distilled water and the UV light absorbance was measured at 280nm. A further 0.2ml of each fraction was added to 5ml NE260 scintillation fluid (Nuclear Enterprises Ltd) and the radioactivity measured by scintillation counting.

RESULTS

CSF Concentrations of Anticonvulsants

The CSF concentrations of phenytoin, phenobarbitone and primidone gave a good correlation with serum concentrations (Figs 8.1a,b,c). The CSF concentrations were found to be on average 12% of the total serum concentrations for phenytoin, 47% for phenobarbitone and 100% for primidone.

Protein Binding of Anticonvulsants In Vivo

The plasma protein binding of phenytoin and phenobarbitone was measured by ultrafiltration in 63 patients receiving one or both of these drugs. The mean percentage free phenytoin in 63 patients was 10.5 ± 2.5 SD; percentage free phenobarbitone in 32 patients was 52.9 ± 5.5 SD.

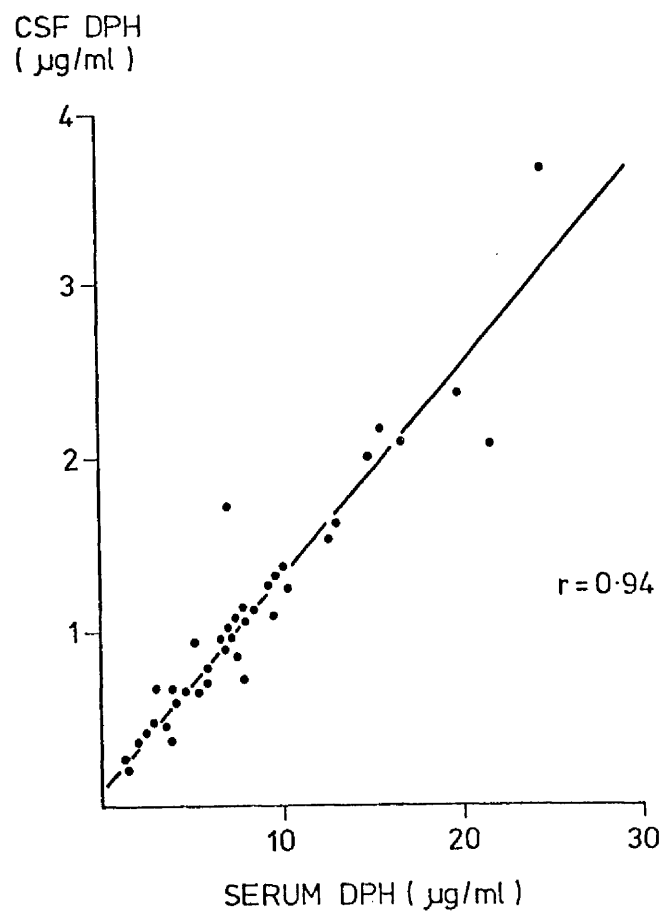


Fig 8.1a Relationship between serum and CSF concentrations of phenytoin in 40 patients

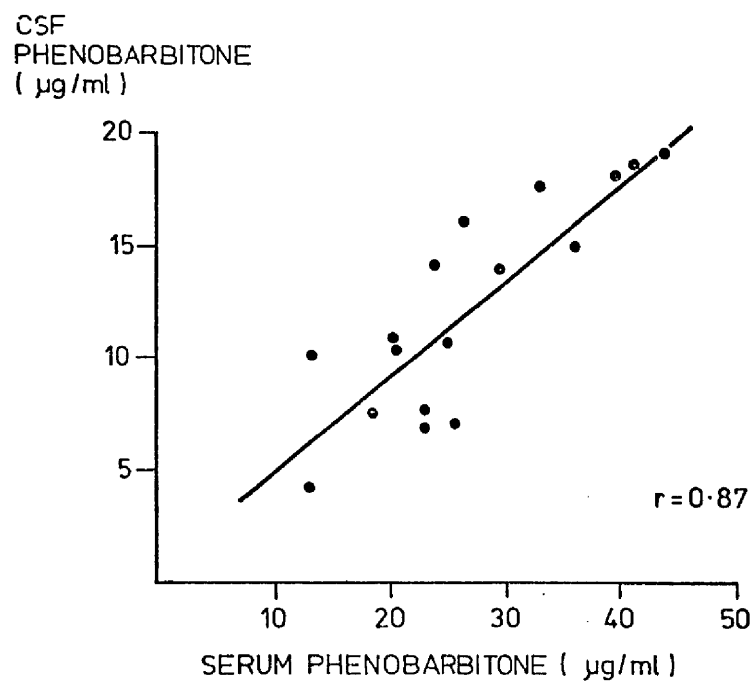


Fig 8.1b Relationship between serum and CSF concentrations of phenobarbitone in 17 patients

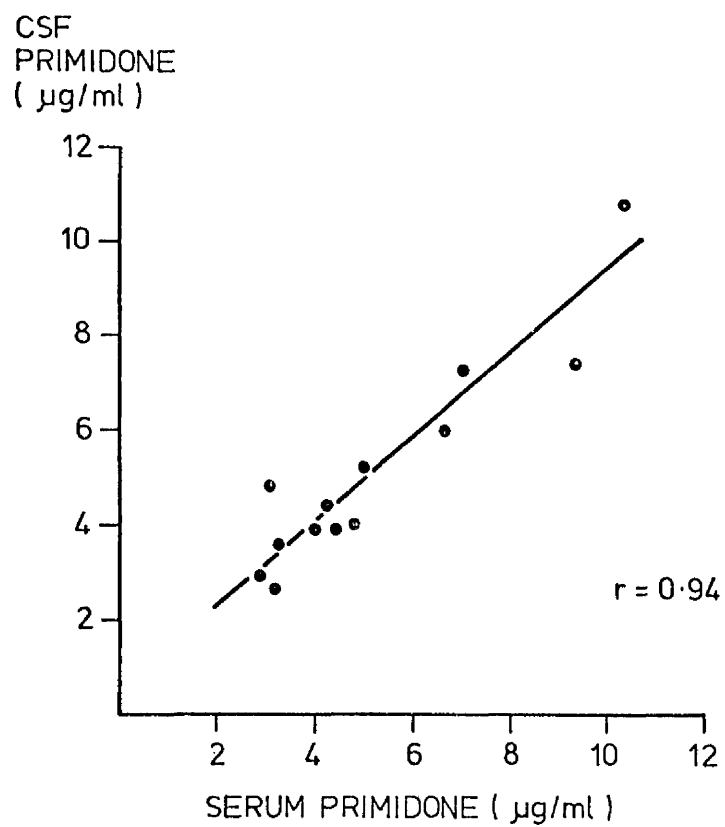


Fig 8.1c Relationship between serum and CSF
concentrations of primidone in 13
patients

Serum albumin concentrations were measured in 28 of these patients. Fig 8.2 shows a graph of percentage free phenytoin plotted against serum albumin level. A significant positive relationship was found ($p < 0.05$), suggesting that patients with reduced albumin levels show reduced binding of phenytoin. There was no significant relationship between age and binding of phenytoin or phenobarbitone in the patients described above, although there was a trend towards reduced binding in the elderly. There was also a trend towards reduced albumin levels with age and it appears likely that phenytoin binding is reduced in the elderly only if there is a reduced serum albumin level.

Effect of Plasma Dilution on Plasma Protein Binding of Phenytoin and Phenobarbitone

This was investigated at 37°C using equilibrium dialysis, adding labelled phenytoin and phenobarbitone such that the concentration of both was negligible to the plasma or diluted plasma. The results are shown in Fig 8.3.

Effect of Increasing Drug Concentrations on Plasma Protein Binding of Phenytoin and Phenobarbitone

This was investigated using both ultrafiltration and equilibrium dialysis through cellulose tubing at room temperature. Phenytoin and phenobarbitone were added at a range of concentrations $0-200\text{ }\mu\text{mol/l}$; small concentrations of the labelled drugs were then added. There was no effect of increasing drug concentration on protein binding over this range. The mean percentage bound phenytoin and phenobarbitone measured by the ultrafiltration technique were 88.5 and 50 and by the equilibrium dialysis technique they were 82.5 and 33 respectively.

Effect of Temperature on Plasma Protein Binding of Phenytoin and Phenobarbitone

The protein binding of phenytoin and phenobarbitone (at a plasma concentration of $20\text{ }\mu\text{g/ml}$ of each) was measured in vitro by equilibrium dialysis at three temperatures, 2° , 22° and 37°C . The results are shown in Fig 8.4. The binding of phenytoin was strongly temperature dependent within this range and decreases with increasing temperature,

Free phenytoin
in serum

n = 28

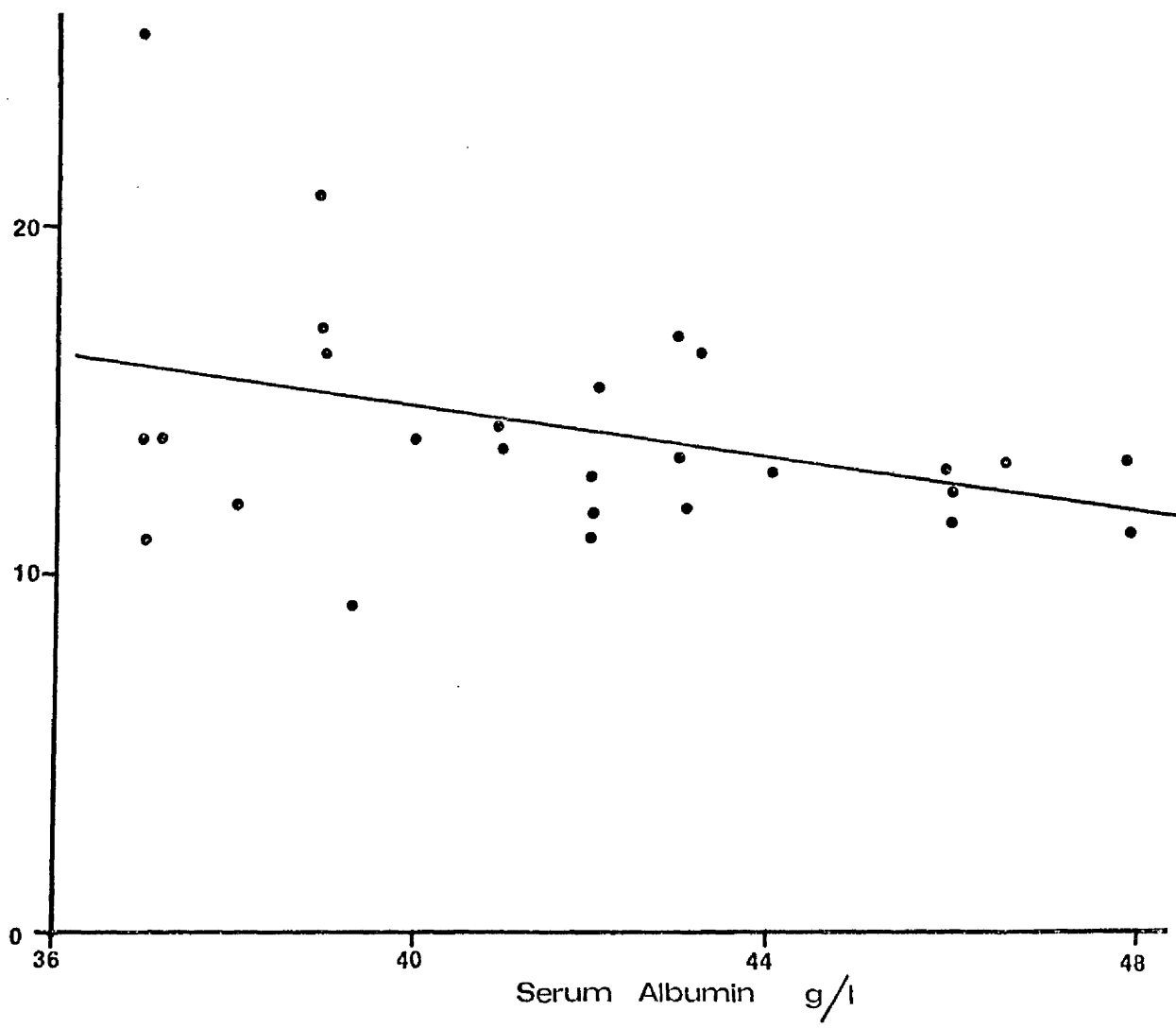


Fig 8.2 Relationship between percentage free phenytoin in serum and the serum albumin concentration in 28 patients. A significant negative correlation ($p < 0.05$) is found

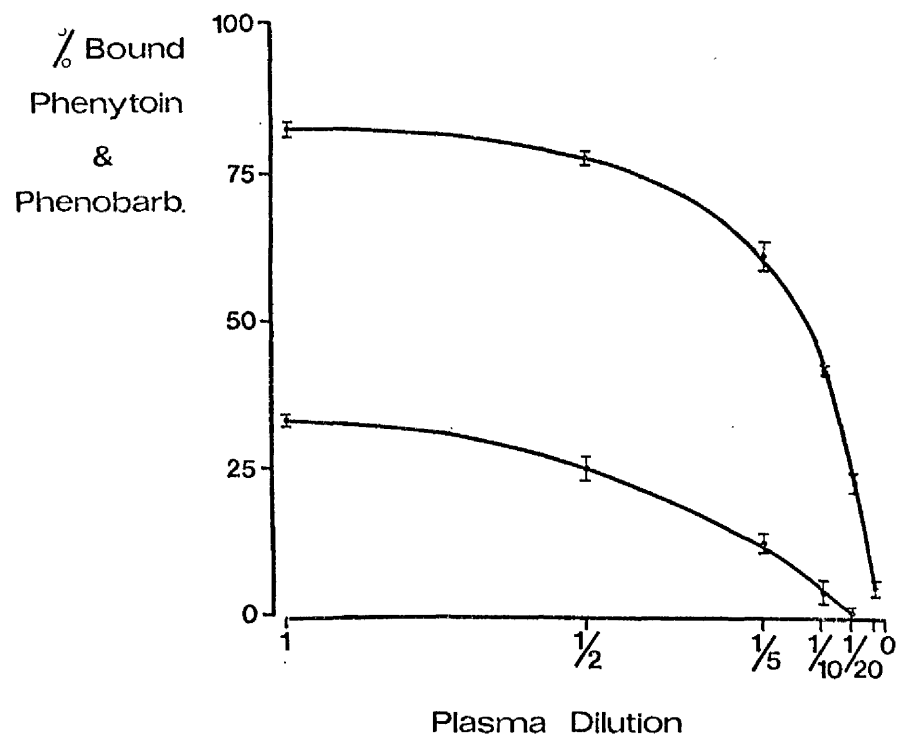


Fig 8.3 Effect of plasma dilution on protein binding of phenytoin and phenobarbitone measured by equilibrium dialysis

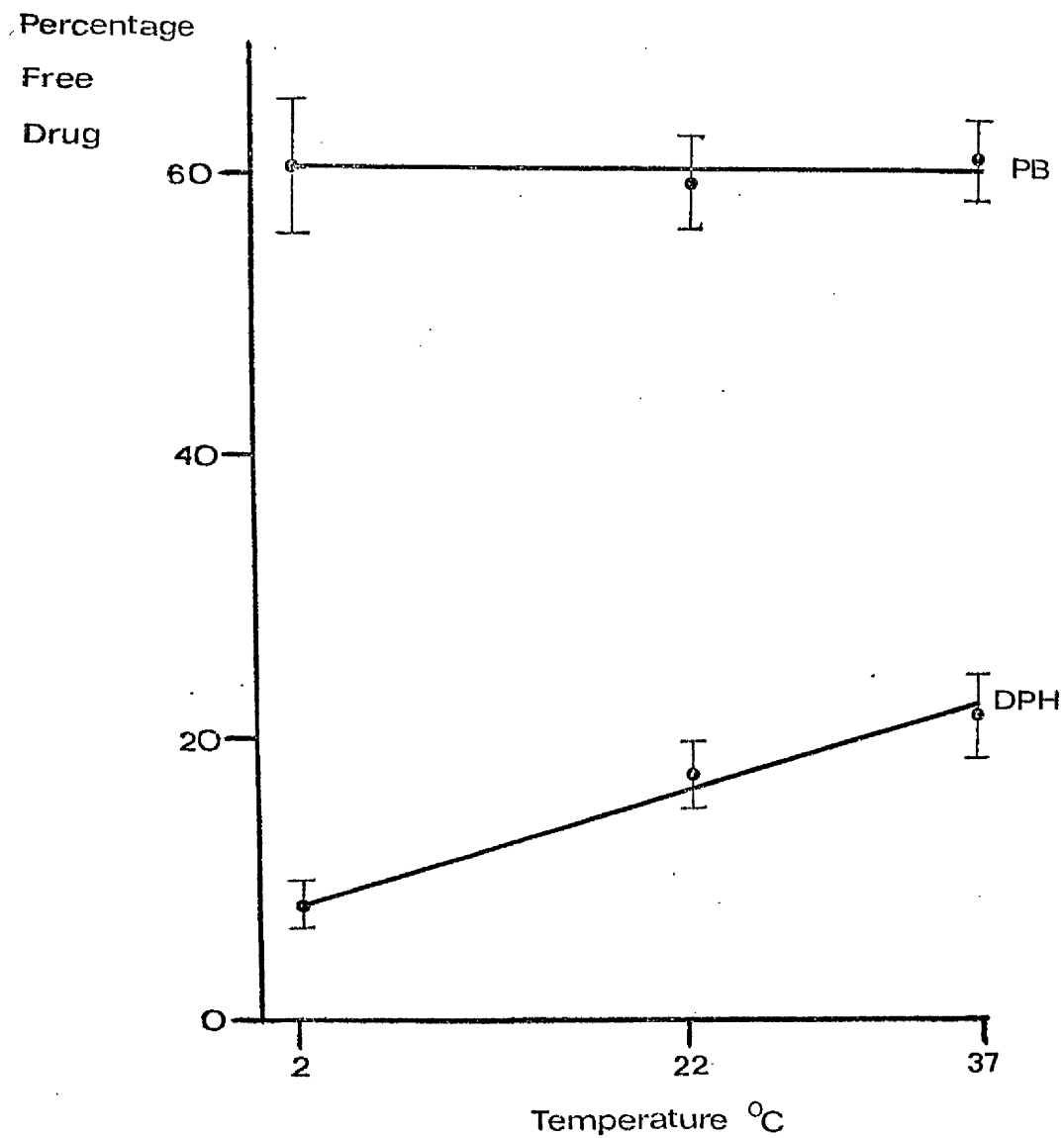


Fig 8.4 Effect of increasing temperature on protein binding of phenytoin and phenobarbitone measured by equilibrium dialysis

the free concentration being 21% greater at 37°C than at 22°C. There was no significant variation in binding of phenobarbitone over the temperature range 0-37°C.

Effect of Sodium Valproate on Protein Binding of Phenytoin and Phenobarbitone

Fig 8.5 shows the results of an initial in vitro study to determine the possible displacing effects of sodium valproate on binding of phenytoin and phenobarbitone. Binding was determined using an Amicon ultrafiltration cell and the drug concentrations in the ultrafiltrate were measured by radioassay, using a double labelling method (see Appendix). The drugs were added to 10ml plasma in the ultrafiltration cell such that a concentration of 20 µg/ml of each was obtained. The drugs were allowed to equilibrate and then sodium valproate was added, to give a concentration of 100 µg/ml. The percentage bound phenytoin decreased from 90.7 to 83.7 and the percentage bound phenobarbitone decreased from 51.7 to 43.7.

Sephadex Fraction of Protein Bound Protein

The results are shown in Fig 8.6. Plasma proteins separated on Sephadex G-100 are eluted as two protein fractions, of which the first is the globulin and the second the albumin fraction. Approximately half the radioactively labelled phenytoin was found by this technique to be bound to the albumin fraction.

DISCUSSION

In previous studies, four methods have been generally used to determine phenytoin binding to plasma proteins : measurement of the ratio of CSF to total plasma phenytoin concentrations; equilibrium dialysis; ultrafiltration with cellulose tubing and ultrafiltration with non-cellulosic polymeric membrane (Amicon) filtration cones (Table 8.1). The latter technique was not used in this study as other workers have shown that these non-cellulosic ultrafiltration cones permit the leakage of 2-6% of the albumin into the filtrate (Porter and Layzer, 1975) and further error

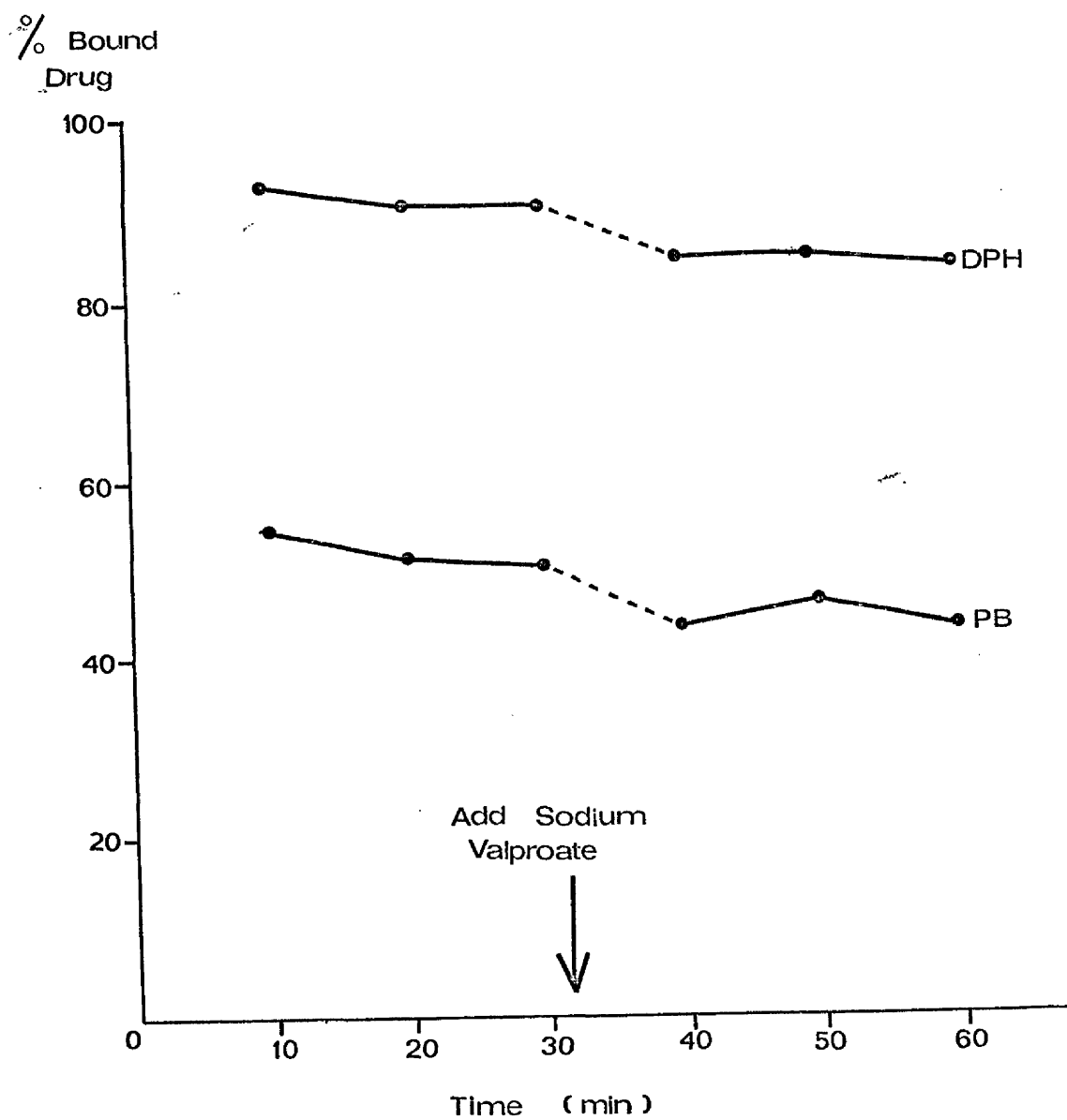


Fig 8.5

Effect of sodium valproate (100ug/ml) on plasma protein binding of phenytoin and phenobarbitone. This was measured in vitro using an Amicon ultrafiltration cell

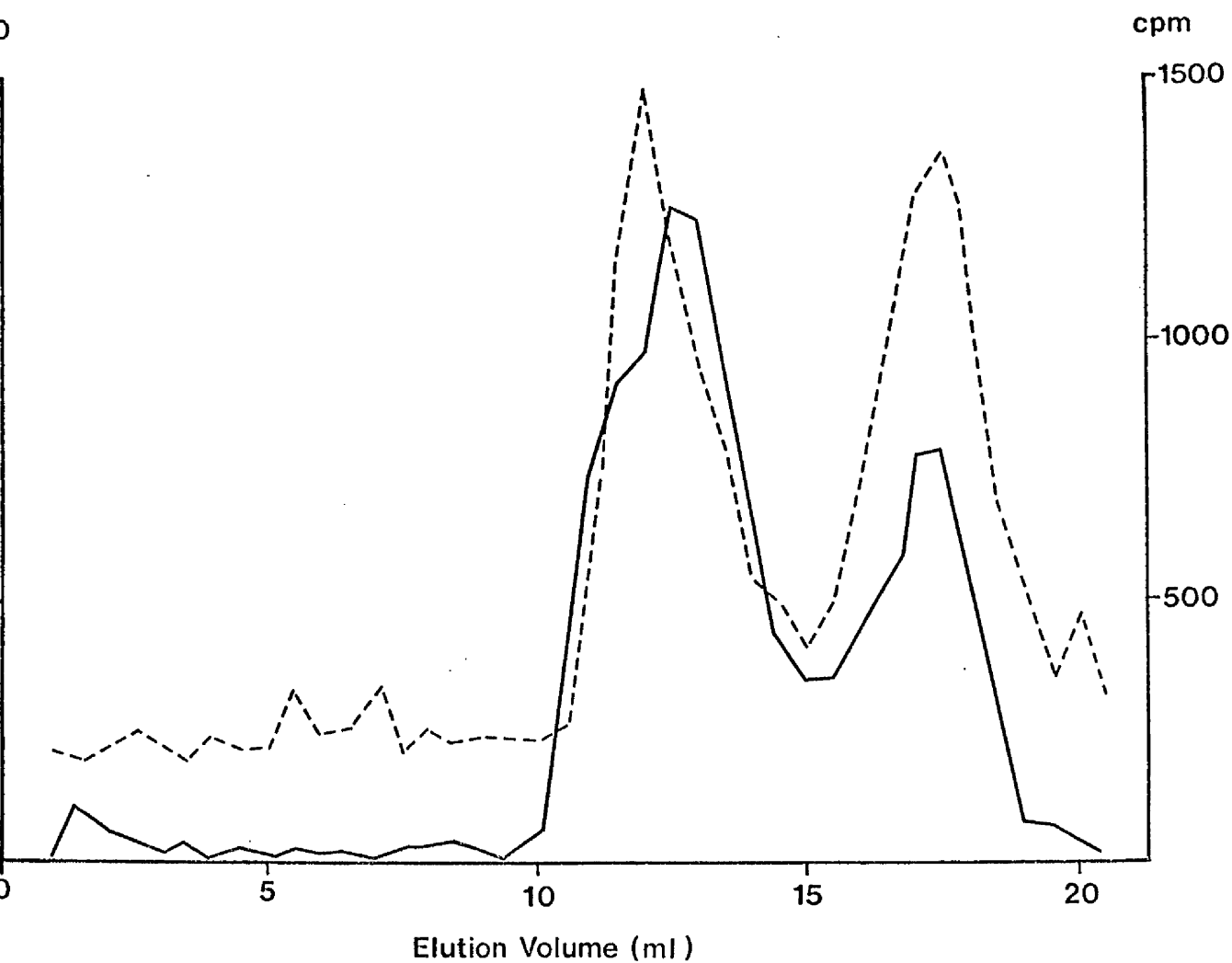


Fig 8.6 Sephadexfractionation of ¹⁴C-labelled phenytoin bound to plasma proteins. The protein concentration in the fractions collected was measured by the UV absorbance at 280nm: protein concentration is shown by the uninterrupted line (—). ¹⁴C-phenytoin was measured by scintillation counting and is shown by the interrupted line(----)

would be expected from the fact that the membranes bind 7% of phenytoin in the unfiltered plasma (Shah et al, 1974). However, a related technique using an Amicon ultrafiltration cell offers a rapid method for examining possible displacing effects of drugs and has been used for study of the effect of sodium valproate on protein binding of phenytoin and phenobarbitone in vitro.

In the present study the protein binding of phenytoin was measured directly by ultrafiltration and equilibrium dialysis through cellulose tubing and the values obtained were similar to those found by other workers (Table 8.1), with the equilibrium dialysis technique showing higher free concentrations as has been described elsewhere (Lunde, 1970; Kurata and Wilkinson, 1974). While measurement of the free concentration of the drug in plasma is preferable to measurement of the total concentration, such an assay involves additional methodologic complexity. As CSF is in effect an ultrafiltrate of plasma (its protein content usually less than 50mg/100ml, phenytoin concentration in CSF has been taken as a measure of the free concentration (Triedman et al, 1960; Viukari and Tammisto, 1959; Lund et al, 1972). The present study shows that CSF concentrations of phenytoin and phenobarbitone do indeed appear to reflect free concentrations; primidone is unbound in plasma and CSF concentrations are similar to total plasma concentrations. Obviously lumbar punctures cannot be performed routinely only for the purpose of measuring protein binding on drugs. However, a related method which may be suitable for routine purposes is the measurement of drug concentrations in saliva; a study of salivary concentrations of phenytoin is described in the following Chapter.

There were significant variations in the free fraction of phenytoin between different patients, but the binding of phenobarbitone was relatively constant. There was no relationship between the degree of binding of phenytoin and the total serum concentration of the drug and in vitro studies showed that there was no significant variation in the binding over the concentration range 0-200 $\mu\text{mol/l}$.

There was, however, a significant trend towards lowered binding in patients with lower plasma concentrations of albumin although there was no relationship between phenytoin binding and total protein concentration. Other workers have found a significant relationship between albumin level and plasma phenytoin binding (Porter and Layzer, 1975) but a lack of dependency on total serum protein concentration has also been reported (Olsen et al, 1975). The effect of a reduced albumin level would appear to be surprisingly large in view of the fact that in vitro studies showed relatively little effect of plasma dilution over a reasonable range. Also, a study of the binding to individual protein fractions by Sephadex fractionation showed that only about half of the protein-bound phenytoin is bound to albumin. Other workers have shown that phenytoin is bound to alpha globulins in addition to albumin (Lightfoot and Christian, 1966). A study of phenytoin binding to particular albumin fractions in uraemic patients (Shoeman et al, 1973) has suggested that a small reduction in total albumin concentrations in particular patients may cause considerably reduced phenytoin binding because of a large reduction in concentrations of the albumin fractions that account for most of the binding. It is possible that a higher proportion of the phenytoin is bound to albumin at 37° - Sephadex fractionation in this study was carried out at room temperature. The variation in binding of phenytoin should also be taken into account in comparing protein binding measured by equilibrium dialysis or ultrafiltration, at room temperature with the protein binding measured as the CSF/serum ratio. A mean of 10.5% of phenytoin was free at 22° as measured by ultrafiltration; at 37° this would be expected to increase to 14.9%.

In the previous Chapter it was suggested that sodium valproate may displace phenytoin from its plasma protein binding sites. An initial in vitro study described here suggested that this may indeed be the case and that sodium valproate may also displace phenobarbitone. This was confirmed in an in vitro study by other workers (Jordan et al, 1976) and an in vivo study in rats (Patsalos and Lascalles, 1977).

has also suggested the possible importance of a displacing effect of sodium valproate on phenytoin binding. There is, however, still a need for a study of the importance of this effect in the clinical situation.

Determination of free levels of drugs in plasma in individual patients is of value if the degree of protein binding determines the degree of entry of the drug into the brain, and hence the therapeutic and toxic effects. This was suggested to be the case at least for phenytoin by a study in rats; phenylbutazone was found to increase the potency of phenytoin by displacing it from plasma protein (Shoeman and Azarnoff, 1975). No study in human patients has yet been carried out to demonstrate that the variation in protein binding is indeed of clinical significance, but it seems likely that this will be found to be the case. It is particularly important to study the protein binding of drugs such as phenytoin which are highly bound, since it would be expected that they would show particularly wide degrees of variation. There is a need for in vivo studies of the protein binding in human patients of the new anticonvulsants clonazepam and sodium valproate. Variations in protein binding might alter the therapeutic effectiveness of these drugs and particularly clonazepam, which is present in low concentrations, might be susceptible to displacement from its binding sites by other drugs. Displacement by phenytoin and phenobarbitone of clonazepam from plasma proteins would be another explanation of the increased toxic effects of clonazepam in patients on multiple drug therapy (described in Chapter 6) and this would also be expected to lower serum concentrations of clonazepam, as was found. However, this would not explain the reduced therapeutic effectiveness of clonazepam in patients on multiple drug therapy.

SUMMARY

1. The plasma protein binding of phenytoin shows significant differences between different patients and this may be of therapeutic importance in altering tissue levels of the drug.

2. Binding of phenytoin to different plasma protein fractions was studied by Sephadex fractionation. Approximately half the bound phenytoin was bound to the albumin fraction .
3. Protein binding of phenytoin was lower in patients showing low serum albumin concentrations but a relatively large plasma dilution was required to produce a comparable effect.
4. Protein binding of phenytoin but not phenobarbitone was lower at 37°C than at room temperature.
5. Sodium valproate was found to displace phenytoin and phenobarbitone from their plasma protein binding sites in vitro.

CHAPTER 9

THE VALUE OF MEASURING PHENYTOIN IN SALIVA

INTRODUCTION

Chapter 8 has shown that plasma protein binding of phenytoin may vary widely in different patients. However, the techniques which were used to measure free levels of phenytoin - equilibrium dialysis, ultrafiltration, measurement of CSF concentrations - are unsuitable for routine use. It has been suggested that salivary phenytoin concentration is dependent on the free concentration (Bochner et al, 1974) and this offers a readily obtainable measure of the biologically available drug.

A new radioimmunoassay technique has recently been produced which is sufficiently sensitive to measure low concentrations of phenytoin in small quantities of fluid (Paxton et al, 1977). In this Chapter the value of this technique in the measurement of saliva concentrations of phenytoin has been studied and the results for saliva concentrations have been compared to concentrations in serum and CSF.

METHODS

Simultaneous samples of blood and saliva were obtained from 104 in-patients for analysis of phenytoin. In addition, CSF was obtained from 33 of these patients. All patients were receiving phenytoin and in most cases one or more other drugs, usually phenobarbitone or primidone. Blood samples were allowed to clot, serum separated and stored at -20°C . CSF and saliva specimens were deep frozen until assayed. The mixed saliva samples were centrifuged at 2500g for 15 minutes to remove any debris. Phenytoin in serum, saliva and CSF was measured by a new radioimmunoassay technique (Paxton et al, 1976) (see Appendix) which is more sensitive than existing

methods and requires only 20 μ l of fluid. Phenytoin concentrations in serum were also measured by GLC; there was good correlation between the GLC and radioimmunoassay methods for assaying phenytoin in serum ($r = 0.95$). Measurements of phenytoin by this radioimmunoassay technique were carried out by Dr J W Paxton in the Department of Materia Medica.

RESULTS

The mixed salivary concentrations of phenytoin gave a good correlation with serum concentrations in 104 patients ($r = 0.93$, the equation of the line being saliva concentration = $-0.002 + 0.095$ serum concentration) and an even better correlation with CSF concentrations in the 33 patients in whom lumbar punctures were performed ($r = 0.98$, the equation of the line being saliva concentration = $0.03 + 0.80$ CSF concentration). The relationship between saliva and CSF concentrations is shown graphically in Fig 10.1 and the results are summarised in the Table below.

Fraction Ratios	No of Patients	Range (%)	Mean Ration (%)	SD
Mixed salivary conc : Total serum conc	104	5.1-18.3	9.6	2.6
CSF conc : Total serum conc	33	9.8-25.0	12.0	2.8
Mixed salivary conc : CSF conc	33	66-111	82.3	11.3

Table 9.1 Phenytoin concentration ratios in epileptic patients

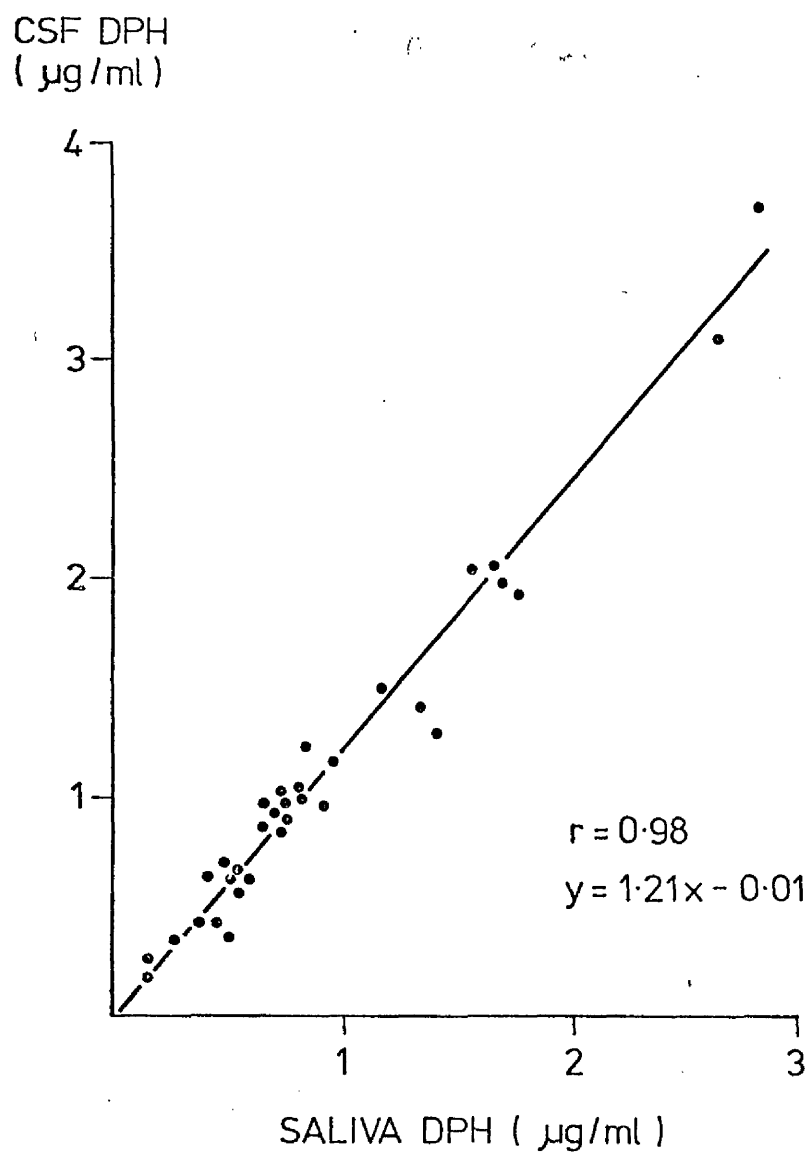


Fig 9.1 Relationship between saliva and CSF concentrations of phenytoin

DISCUSSION

The previous Chapter described assay of phenytoin in CSF, which is in effect an ultrafiltrate of plasma, as a measure of the free concentration. This approach is not suitable for routine use, but it is known that the concentrations of drugs in saliva (mean protein content 262mg/100ml) may also reflect the free concentrations in plasma. Bochner et al (1974) have measured phenytoin concentrations in saliva and they found that they were similar to plasma free concentrations measured by ultrafiltration. However, saliva concentrations were measured by a gas chromatography technique which required volumes of at least 2ml. Volumes as large as this may be difficult to obtain from all but the most co-operative of patients and in this study a radioimmunoassay technique has been used to measure phenytoin salivary concentrations in microlitre volumes. The correlation found between saliva, CSF and serum concentrations would be expected to be of value in situations, such as uraemia, where serum protein binding is abnormal. In addition, the technique is non-invasive and may be valuable in paediatric practice and in pharmacokinetic studies in which multiple venepunctures would otherwise be required.

When this work was begun, literature regarding the correlation between plasma and saliva levels for phenytoin was fragmentary. Svensmark et al (1960) reported no relation between the concentration of phenytoin in serum and saliva, and Ciancio et al (1972) found a significant correlation only at low serum and salivary concentrations of the drug. Recently, other studies have confirmed that measurement of phenytoin concentrations in saliva is of value. Other workers have found that saliva levels give an accurate indication of phenytoin available in CSF (Troupin and Friel, 1974; Schmidt and Kupferberg, 1975) and of free phenytoin in plasma (Reynolds et al, 1976) and there has been another study in which phenytoin was measured by a radioimmunoassay technique in small

volumes of saliva (Cook et al, 1975). Primidone, phenobarbitone, carbamazepine and ethosuximide concentrations in saliva have also been shown to reflect plasma free concentrations (Horning et al, 1977; McAuliffe et al, 1977) but salivary concentrations of sodium valproate are much lower than expected because the transfer into saliva is pH-dependent and this drug has a low pKa.

SUMMARY

1. A new radioimmunoassay technique has been used for the measurement of phenytoin in small volumes of saliva.
2. Highly significant correlations were found between phenytoin concentrations in serum and saliva ($r = 0.93$) and particularly between CSF and saliva ($r = 0.98$). Assay of phenytoin concentration in saliva offers a simple measure of the plasma free concentration.

CHAPTER 10

UPTAKE OF DRUGS BY RED BLOOD CELLS AS A MEASURE OF BIOLOGICALLY AVAILABLE DRUG

INTRODUCTION

In Chapter 8 it was shown that the degree of plasma protein binding of phenytoin may vary between different patients and it might be desirable to measure protein binding routinely in the clinical situation; in the previous Chapter a simple method for measuring protein binding of phenytoin was described, using a radioimmunoassay technique to measure concentrations of phenytoin in saliva. However, this radioimmunoassay technique is not widely available and also it might be desirable to have a method which could be readily adapted to the measurement of free concentrations of other anticonvulsants; it would of course be possible to measure saliva concentrations by GLC (McAuliffe et al, 1977) but the fairly large volumes of saliva required may be difficult to obtain from any but the most obliging of patients.

Kurata and Wilkinson (1974) have suggested that determination of the RBC/plasma concentration ratio may serve as a simple and rapid technique for the screening of abnormal protein binding in routine clinical samples. The assumption is that the RBC concentration depends on the free rather than the total concentration in plasma. This Chapter describes a method whereby the uptake of phenytoin and phenobarbitone can be determined simultaneously using doubly labelled radioisotopes. In addition, whole blood concentrations of these drugs and of primidone and PEMA have been measured by GLC.

PATIENTS AND METHODS

RBC Binding of Anticonvulsants In Vivo

1. Heparinised blood was obtained from a group of epileptic patients receiving one or more of the anti-convulsants phenytoin, phenobarbitone or primidone. Gas-liquid chromatography was used to determine anti-convulsant concentrations in whole blood and in plasma. In 6 patients PEMA concentrations were also measured.
2. The erythrocyte/plasma concentration ratios for phenytoin and phenobarbitone in 41 patients were obtained by a radioassay technique (see Appendix) after adding small concentrations of ^3H -labelled phenytoin and ^{14}C -labelled phenobarbitone to whole blood. After equilibration for 30 minutes at 22°C , the plasma was removed and the drug concentrations determined by scintillation counting. In order to determine the uptake of these drugs by RBC's from buffer, they were washed twice in their own volume of phosphate buffer and the packed cells resuspended to twice their own volume. 0.1ml of the previous washing and of the resuspended RBC's were taken for counting. The uptake of the drugs by RBC's from plasma is calculated using the equation

$$\frac{c}{p} = \frac{B/P - (1 - H)}{H}$$

where C is the red blood cell concentration, B is the known whole blood concentration, P is the plasma concentration and H is the haematocrit.

RBC Binding of Anticonvulsants In Vitro

Heparinised blood was obtained from normal volunteers and centrifuged and the plasma and buffer layers removed. The RBC's were washed twice with isotonic phosphate buffer (K_2HPO_4 1.41, NaH_2PO_4 0.26, NaCl 18.10g/l : pH 7.4) and the packed RBC's resuspended to twice their own volume with varying dilutions of plasma containing 20 $\mu\text{g/ml}$ each of radioactively labelled phenytoin and phenobarbitone. After equilibration, for thirty minutes, the drug

concentrations in plasma and RBC's were measured by scintillation counting.

RESULTS

Concentrations of phenytoin, phenobarbitone, primidone and PEMA were determined by GLC in plasma and whole blood, and the concentrations in RBC's were determined by subtraction. Primidone and PEMA are unbound in plasma and RBC concentrations were approximately equal to plasma concentrations. The results are shown in Table 10.1 below.

DRUG	No of Patients	m	b	r
Phenytoin	40	0.755	1.4	0.924
Phenobarbitone	40	0.738	12.5	0.859
Primidone	17	0.92	5.5	0.948
PEMA	6	0.90	3.9	0.947

Table 10.1 Relationship between whole blood and plasma concentrations of phenytoin, phenobarbitone, primidone and PEMA according to the equation $y = b + mx$, where y is whole blood concentration and x is plasma concentration in $\mu\text{mol/l}$ and r is the correlation coefficient

Primidone and PEMA are unbound in plasma and the values obtained for RBC concentrations by subtraction suggest that there is a simple equilibration between plasma and RBC's, with the concentrations being approximately equal.

The uptake of phenytoin and phenobarbitone by RBC's was determined by a radioassay technique. The uptake of these drugs from isotonic phosphate buffer was also determined. The results are shown in Table 10.2.

DRUG	RBC/PLASMA (a)	RBC/BUFFER (b)	a/b
Phenytoin	0.58 \pm 0.08	2.8 \pm 0.3	0.21 \pm 0.3
Phenobarbitone	0.88 \pm 0.08	0.99 \pm 0.19	0.88 \pm 0.13

Table 10.2 Uptake of phenytoin and phenobarbitone
by RBC's obtained from 41 patients.
All values are means \pm standard
deviation

It would be expected that the ratio a/b would be approximately equal to the free fraction of these drugs in plasma. In fact, the values obtained for this ratio either for phenytoin or phenobarbitone are somewhat higher than the values obtained by ultrafiltration for the protein free drug (described in Chapter 8). This may be explained by the swelling of the RBC's in buffer, in the absence of protein, which may reduce the uptake of the drug.

That the RBC concentration is related to the free rather than the total concentration can be shown by the effect of plasma dilution. Fig 10.1 shows the effect of plasma dilution on the uptake of phenytoin and phenobarbitone by RBC's. Comparing this figure to Fig 8.3, in which the effects of plasma dilution on protein binding are shown, it can be seen that the RBC uptake increases as protein binding decreases.

DISCUSSION

Phenytoin, phenobarbitone, primidone and PEMA all accumulate in human RBC's, and uptake appears to be a function of the concentration of unbound drug in the plasma. A constant RBC/unbound plasma concentration partition coefficient with little interindividual variability exists. Perturbations in the unbound fraction of phenytoin and phenobarbitone produced by plasma dilution do not appear to affect the coefficient. Such results have been found previously for

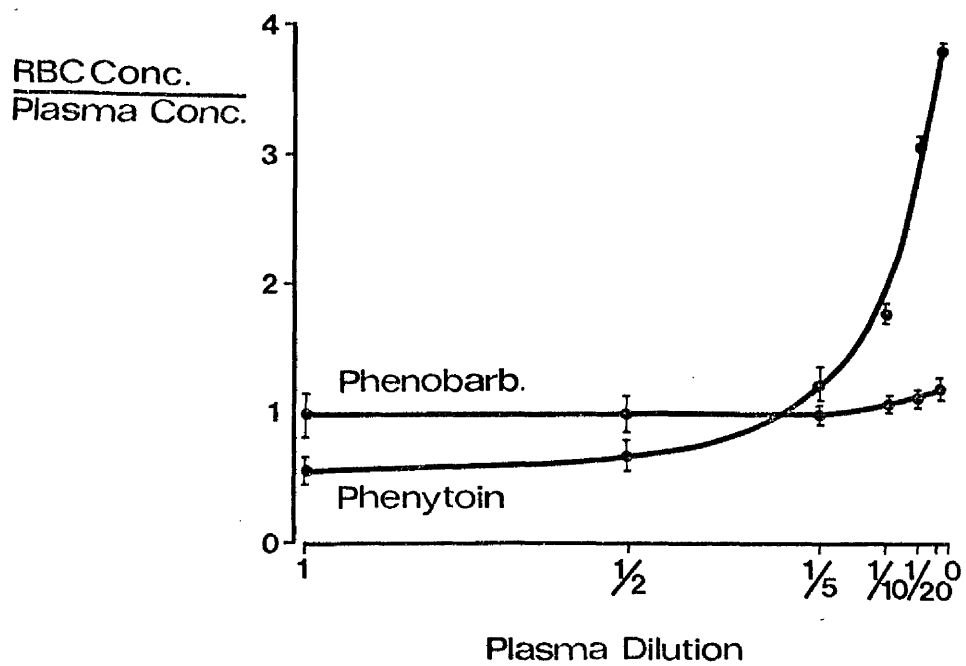


Fig 10.1 Effect of plasma dilution on the RBC uptake from plasma of phenytoin and phenobarbitone

phenytoin (Borondy et al, 1973; Kurata and Wilkinson 1974; Sherwin et al, 1976) propranolol (Jellett and Shand, 1974, quinidine (Hughes et al, 1975) and haloperidol (Hughes et al, 1976). Direct measurement of the phenytoin content of RBC's by the method described here may provide a useful index of percentage free drug in plasma. However, the protein binding of phenobarbitone varies only slightly between different patients and there would be little value in measuring RBC uptake of this drug. It should be noted that because of the variation in plasma protein binding of phenytoin with temperature, the uptake of the drug by RBC's varies with temperature. If there is no effect of temperature over the range 22°C - 37°C on the uptake of the free drug by the RBC and, as was shown in Chapter 8, the free fraction of drug in plasma increases over this temperature range, then it would be expected that more of the phenytoin in a blood sample will be bound in the RBC's at 37°C than at 20°C . This is of importance since blood samples are normally spun down and the plasma or serum removed for anticonvulsant analysis at room temperature. The phenytoin in plasma or serum will be greater at room temperature than in the in vivo situation, since there will be less bound in the RBC's. If the haematocrit is 0.50, it can be calculated that the measured serum concentration will be approximately 10% greater than the in vivo concentration. This has also been suggested recently by other workers (Ehrnebo and Odar-Cederlöf, 1977). Thus the CSF/serum or saliva/serum ratios as measured will be 91% of the true values. This may at least partly explain why the values for protein binding obtained in Chapter 8 by equilibrium dialysis or ultrafiltration were greater than the values obtained for the CSF/serum or saliva/serum phenytoin concentration ratios. The true mean CSF/serum ratio obtained from the results described in Chapter 8 would then be 13.3, which compares reasonably well with a percentage free phenytoin at 37° measured by ultrafiltration of 14.9.

SUMMARY

1. Phenytoin, phenobarbitone, primidone and PEMA concentrations in whole blood from epileptic patients have been measured by GLC. In all cases the whole blood concentration was directly proportional to the plasma concentration.
2. A simple radioassay technique has been developed for the simultaneous determination of uptake by RBC's of phenytoin and phenobarbitone. This provides a readily available index of the percentage free drug in plasma.
3. Uptake of phenytoin by RBC's does not vary with temperature. However, since protein binding decreases with increasing temperature, a higher concentration of the drug is bound in the RBC's at 37°C than at 22°C.

CHAPTER 11

ANTICONVULSANTS AND FOLATE METABOLISM

INTRODUCTION

'Folate' is a general term for a group of pteridines which are required in the diet for normal metabolism. Deficiency of folate leads to the development of megaloblastic anaemia, morphologically identical to that of vitamin B₁₂ deficiency. Investigations of megaloblastic anaemias not associated with low serum vitamin B₁₂ showed that certain of them were related to anticonvulsant therapy and that the effect was due to a secondary fall in serum folate (Kohn et al, 1961; Druskin et al, 1962). When other workers studied groups of non-anaemic patients receiving anticonvulsants, however, it became apparent that low serum folate concentrations were present in a large percentage of the epileptic population varying from 27% to 91% (reviewed by Reynolds, 1973). Other studies have shown that sub-normal serum folate levels are accompanied by corresponding lowering of RBC folate (Preece et al, 1971) and of CSF (Wells and Casey, 1967; Reynolds et al, 1969, 1972).

The clinical importance of the lowered serum folate concentrations is not clearly defined. There have been various reports of worsening of seizures in patients who received therapeutic administration of folate (Chanarin et al, 1960; Reynolds, 1967; Wells, 1968; Dennis and Taylor, 1969; Lankowsky et al, 1969), but a series of controlled trials to study the effect of folic acid administration on seizure control all produced negative results (Grant and Stores, 1970; Jensen and Olesen, 1970; Ralston et al, 1970; Norris and Pratt, 1971; Mattson et al, 1973). However, Mattson et al (1973) found no rise in CSF folate during folic acid administration and suggested that anticonvulsants interfere with conversion of folic acid to

5-methyltetrahydrofolate, which is the form of folate which enters the CSF. Administration of 'Leucovorin' (5-formyltetrahydrofolate) to a group of six patients produced a significant rise in CSF folate but there was no change in seizure frequency. 5-formyltetrahydrofolate is a reduced folate which is rapidly converted to 5-methyltetrahydrofolate (Nixon and Bertino, 1972).

Low folate concentrations have been related to treatment with the three major anticonvulsant drugs (phenytoin, phenobarbitone and primidone) and Reynolds et al (1966) found that the incidence rose with the number of drugs that the patient was receiving. However, Klipstein (1964) reported that the incidence of low concentrations was the same in patients on phenytoin alone as in patients on phenytoin plus other drugs. The relationship between lowered folate and the anticonvulsant regime remains unclear and therefore this Chapter describes measurements of serum folate in patients on various drug therapies in order to study the relative effects. In addition, a double-blind trial has been conducted in which the effects of folic acid and 'Leucovorin' administration on serum and CSF concentrations of folate, serum concentrations of anticonvulsants and seizure control have been assessed and compared.

The effects of anticonvulsant and folate administration on concentrations in CSF of 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), the acid metabolites of serotonin and dopamine respectively, have also been studied. It is known that pharmacological agents that decrease endogenous brain amines increase the susceptibility to experimental seizures, while agents that increase biogenic amines decrease the susceptibility to convulsions. There is a possibility that the action of anticonvulsant drugs may involve an effect on the brain amines, and this may be reflected in the CSF concentrations of HVA and 5-HIAA. Much evidence supports the view that the concentrations of these metabolites reflect the turnover of their parent amines (Moir et al, 1970). Folate may be involved in

the metabolism of these amines (Laduron, 1972; Banerjee and Snyder, 1974) and it is possible that an effect of anticonvulsants on brain amine metabolism may be mediated via an effect on tissue folate. It would in that case be expected that administration of folate would reverse the biochemical effect of anticonvulsants and worsen seizure control.

PATIENTS AND METHODS

The subjects studied were 61 male and 50 female epileptic in-patients, with ages ranging from 18 - 66 years (mean 37 years). All patients had been receiving various combinations of phenytoin, phenobarbitone and primidone for at least one year. In addition some patients were receiving other anticonvulsant drugs - carbamazepine (17), sodium valproate (11), clonazepam (5), valium (5), sulthiame (2) and ethosuximide (1). Blood samples for analysis of serum folate and anticonvulsant concentrations were obtained from all patients on at least one occasion and in 75% of patients on two or more occasions. In addition, serum B₁₂ concentrations were measured in 103 of the patients.

Lumbar punctures were performed for analysis of CSF concentrations of folate from 43 patients; in addition to folate, CSF concentrations of HVA (24 patients) and 5-HIAA (30 patients) were measured. Nineteen patients who showed low CSF concentrations of folate were selected for a double-blind trial of the effects on seizure control of administration of folic acid and 'Leucovorin'. All these patients had low levels of serum folate also. Fourteen patients were receiving phenytoin combined with phenobarbitone or primidone, four patients were receiving phenobarbitone or primidone and one patient was receiving phenytoin alone. Folic acid (5mg intramuscular daily) and 5-formyltetrahydro-folate (3mg intramuscular daily) were administered for a period of one month, after which time a second lumbar puncture was performed. Ten patients received 'Leucovorin'; and nine patients received folic acid.

CSF concentrations of folate, HVA and 5-HIAA were measured both before and after folate administration. Serum concentrations of folate and anticonvulsants (phenytoin, phenobarbitone, primidone and PEMA) were measured at the same time as lumbar punctures were performed and in addition serum folate concentrations were measured three months after the start of folate therapy. Seizures were recorded by the nursing staff as major or minor seizures and the frequencies were compared for three months before the trial and three months after the start of folate therapy.

RESULTS

Serum folate concentrations in 111 patients receiving various combinations of anticonvulsant drugs are summarised in Table 7.1. 73% of patients were found to have folate concentrations below the normal range (3.2 - 17 $\mu\text{g/l}$). There was no significant difference between single treatment with phenytoin and treatment with phenobarbitone or primidone, and there was no significant difference between single treatment and combined treatment with these drugs. However, higher serum concentrations of folate were found in patients on low doses of phenobarbitone only - five patients with doses in the range 60 - 120mg daily showed a mean of 5.2 $\mu\text{g/l}$ and the concentrations were significantly higher ($p < 0.001$, t-test, comparing the log serum concentrations) than in other patients receiving anticonvulsant therapy. Also, there appeared to be a trend towards lower folate concentrations with higher phenobarbitone serum concentrations in the 11 patients on phenobarbitone alone ($r = 0.672$, $p < 0.05$, correlating log folate concentrations with phenobarbitone concentrations).

A small but significant positive correlation ($r = 0.211$, $p < 0.05$) between serum concentrations of folate and serum concentrations of vitamin B₁₂ was found in 103 patients (correlating the logarithms of the concentrations). However, none of the patients showed B₁₂ concentrations

Drug Combination	No of Patients	Mean Serum Folate ($\mu\text{g/l}$)	SD	Range
Phenytoin	9	2.4	1.0	1.0 - 4.5
Phenobarbitone	11	3.6	1.7	1.2 - 6.0
Primidone	13	2.6	0.8	1.5 - 4.8
Phenytoin + Phenobarbitone	41	2.6	0.9	1.4 - 4.8
Phenytoin + Primidone	32	2.7	1.0	0.8 - 5.8
Phenobarbitone + Primidone	4	2.5	0.8	1.8 - 3.6
Phenobarbitone + Primidone + Phenytoin	1	2.6	-	-
Total	111	2.7	1.1	0.8 - 6.0

Table 11.1 Serum folate concentrations in patients receiving various combinations of anticonvulsant drugs

	5-FORMYLTETRAHYDROFOLATE TREATED PATIENTS			FOLIC ACID TREATED PATIENTS		
	No of Patients	Before Treatment	After Treatment	No of Patients	Before Treatment	After Treatment
Serum folate $\mu\text{g/l}$	9	2.2 \pm 0.9	10.8 \pm 3.6	6	2.3 \pm 1.4	10 \pm 1.6
CSF folate $\mu\text{g/l}$	9	8.9 \pm 4.1	41.7 \pm 7.4	6	8.1 \pm 4.2	32.9 \pm 11.1
CSF HVA $\mu\text{mol/l}$	4	0.23 \pm 0.05	0.14 \pm 0.04	3	0.13 \pm 0.03	0.11 \pm 0.05
CSF 5-HIAA $\mu\text{mol/l}$	4	0.13 \pm 0.02	0.05 \pm 0.04	4	0.07 \pm 0.02	0.04 \pm 0.02

Table 11.2

Effects of treatment with folic acid or 5-formyltetrahydrofolate on serum and CSF concentrations of folate and CSF concentrations of HVA and HIAA (mean \pm SD)

below the normal range (160 - 960 pg/ml).

That anticonvulsants lower folate concentrations in CSF as well as in serum was confirmed in the 43 patients in whom lumbar punctures were performed - 65% of patients showed CSF concentrations below the normal range (12.6 - 67 $\mu\text{g/l}$). There was a significant correlation ($r = 0.472$, $p < 0.01$) between serum and CSF concentrations.

Administration of either folic acid or 5-formyltetrahydrofolate raised both serum and CSF levels of folate (with 5-formyltetrahydrofolate having a somewhat greater effect on CSF folate concentrations relative to serum concentrations). These effects are summarised for fifteen patients in Table 11.2 - for four patients on the trial it was not possible to perform a second lumbar puncture and these patients have not been included. Serum folate concentrations were still raised three months after the start of folate therapy (ie two months after the end of folate administration); in nine patients who received folic acid the mean serum folate concentration was 3.9 ± 1.6 SD $\mu\text{g/l}$ after three months as compared to 2.1 ± 1.2 SD $\mu\text{g/l}$ before therapy; in nine patients who received 5-formyltetrahydrofolate (a later blood sample was not obtained from one patient) the concentration was 3.7 ± 1.8 SD $\mu\text{g/l}$ as compared to 2.2 ± 0.7 SD $\mu\text{g/l}$ before therapy.

There was no significant effect of administration of either folic acid or 5-formyltetrahydrofolate on the frequency of either minor or major seizures as shown on Table 11.3 below

Seizures	Folic Acid		5-formyltetrahydrofolate	
	Before	After	Before	After
Major	3.2	2.7	5.2	4.7
Minor	21.8	22.9	7.7	7.9

Table 11.3 Effects of treatment with 5-formyltetrahydrofolate (10 patients) or folic acid (9 patients) on the mean seizure frequencies per month.

However, a significant reduction in serum concentrations of phenytoin ($p < 0.01$, Wilcoxon signed-ranks test) was found as serum folate was raised comparing concentrations of phenytoin before and after one month of folate therapy (Table 11.4 and Fig 11.1). Although there was no overall effect of folate therapy on seizure control there was a possible effect on control of major seizures in patients in whom serum concentrations of phenytoin fell - of nine patients in whom there was a fall in serum concentrations there was an increase in major seizures in five and seizures were unchanged in four (five of these patients were previously not suffering from major seizures, of whom two had major seizures during folate therapy). There were no significant effects of folate therapy on serum concentrations of phenobarbitone, primidone or PEMA.

Brain Amine Metabolism

CSF concentrations of HVA (24 patients) and 5-HIAA (30 patients) were measured in a group of epileptic in-patients receiving chronic anticonvulsant therapy in whom the seizure frequency had been assessed over the previous six months. All patients were receiving phenytoin and in most cases were in addition receiving one or more other drugs, generally phenobarbitone or primidone. The mean phenytoin serum concentration in these patients was $42.2 \mu\text{mol/l}$ (range $6 - 109 \mu\text{mol/l}$). Both HVA and HIAA concentrations were low in these patients - the mean HVA concentration was $0.11 \mu\text{mol/l} \pm 0.11$ (normal range $0.22 - 0.44 \mu\text{mol/l}$) and mean HIAA concentration was $0.11 \mu\text{mol/l} \pm 0.8 \text{SD}$ (normal range $0.8 - 0.3 \mu\text{mol/l}$) - with the HIAA concentrations in 53% of patients showing values at the lowest limit of sensitivity of the assay $0.05 \mu\text{mol/l}$. No correlation was found in these patients between either the HVA or HIAA concentrations and the serum phenytoin concentration, serum or CSF folate concentration, seizure frequency or age of the patients.

DRUG	FOLIC ACID		5-FORMYLtetrahydrofolate	
	No	Before After	No	Before After
Phenytoin	8	52+25 32+10	6	53+22 35+14
Phenobarbitone	4	122+38 143+39	4	111+12 126+52
Primidone		38+18 32+18		36+15 36+ 8
Primidone Phenobarbitone	5	146+63 130+85	4	105+87 128+84
PEMA		31+23 24+19		15+22 43+28

Table 11.4 ✕ Effects of treatment with folic acid or 5-formyltetrahydrofolate
on serum anticonvulsant concentrations

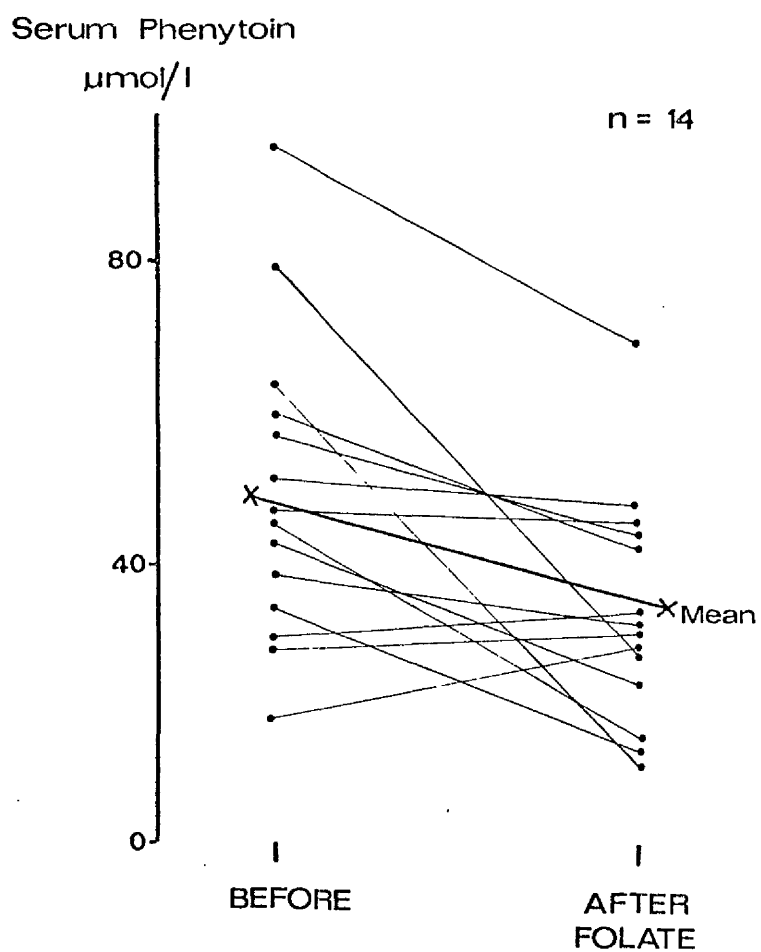


Fig 11.1 Effect of folate therapy on serum phenytoin concentrations in 14 patients. A significant fall ($p < 0.01$) was found

The effects of folate administration as folic acid or 5-formyltetrahydrofolate on CSF concentrations of HVA (7 patients) and HIAA (8 patients) are shown in Table 11.2. There were reductions in concentrations of these metabolites but it is likely that these changes were not significant and they were due to errors in the assay - the measurements were performed on different occasions to determine the levels before and after therapy and there may have been differences in the standardisation of the assay between these two occasions; also there are considerable errors in the assay in measuring the low concentrations of HVA and 5-HIAA found in these patients. However, a definite conclusion can be made that there were no increases in levels of HVA or 5-HIAA with folate therapy.

DISCUSSION

The results in these patients confirm that anticonvulsant administration lowers serum folate concentrations. There was no significant difference in folate concentrations between patients receiving phenytoin and patients receiving phenobarbitone or primidone, although a few patients receiving low doses of phenobarbitone had higher folate levels. Other workers have suggested that phenytoin has a greater effect than these other drugs in lowering serum folate (Reynolds, 1972), but this is probably only the case if the effect of high doses of phenytoin is compared with the effect of low doses of phenobarbitone or primidone. Reynolds et al (1966) found that the incidence of low folate concentrations rose with the number of drugs being taken by the patient but Klipstein (1964) reported that the incidence of low concentrations was the same in patients on phenytoin alone as in patients on phenytoin plus other drugs. In the present study, serum folate concentrations in patients receiving multiple anticonvulsant therapy was only slightly and not significantly lower than in patients receiving single drug therapy, and it appears that, once a low folate level is attained

with a reasonably high dose of a drug, the addition of other drugs has no significant effect. This is similar to results of a study of the effects of administration of phenobarbitone or phenytoin to rats fed on vitamin B₁₂-deficient or vitamin B₁₂-supplemented diets (Williams and Spray, 1976) - the anticonvulsants failed to reduce further the already lowered liver folates resulting from feeding of the vitamin B₁₂-deficient diet, although there was significant depletion in animals taking the supplemented diet.

An inverse correlation between phenytoin and phenobarbitone in serum and folate levels has been found by other workers (Reynolds, 1972b) but in the present study this was found only for phenobarbitone concentrations in patients on low doses of the drug (none of the patients receiving phenytoin or primidone were on comparably low doses of the drug).

The proposed mechanisms for lowered serum folate in patients receiving anticonvulsants include impairment of folate (monoglutamate) absorption (Meynell, 1966). This has not been confirmed (Doe et al, 1971; Fehling et al, 1973), and neither has the hypothesis that phenytoin inhibits the deconjugation of polyglutamates to the glutamate form, which is absorbed (Baugh, 1969). Displacement of folate from plasma protein by anticonvulsants (Markkanen et al, 1973) would lower serum folate but would probably not account for the reduction in RBC folate also observed (Preece et al, 1971). Possibly the most likely hypothesis (Maxwell et al, 1972) is that lowered serum folate is related to an effect of anticonvulsants on the drug-metabolising system in the liver. This possibility is discussed in the following Chapter.

The concentrations of folate in CSF in patients in the present study were also lower than normal, and there was a significant relationship between serum and CSF concentrations of folate. The serum and CSF concentrations were raised both by administration of folic acid and

5-formyltetrahydrofolate. Other workers (Mattson et al, 1973) found that folate concentrations did not rise in CSF in patients who received folic acid although the concentrations rose in serum, presumably because folate must be converted to 5-methyltetrahydrofolate before appearing in the CSF (Levitt et al, 1971). It was assumed that anticonvulsants interfere with this conversion. In conflict with this, folic acid administration did raise CSF concentrations in the present study, although the effect on CSF concentrations compared to serum concentrations was less than with 5-formyltetrahydrofolate administration.

Increase in serum folate concentrations in patients receiving treatment with either folic acid or 5-formyltetrahydrofolate was accompanied by a fall in serum phenytoin concentrations particularly in patients showing previously high concentrations. This confirms previous observations (Jensen and Olesen, 1970; Baylis et al, 1971; Mattson et al, 1973) and may provide an explanation for the conflicting views on the effect of folate therapy on seizure control. It is possible that some of the reports of a worsening of seizures, particularly that by Reynolds (1967), may have been due to the effect of folate in reducing phenytoin concentrations. In the present study, there appeared to be an increase in major seizures in the patients in whom phenytoin concentrations fell and this is in line with the findings in Chapter 4 that increase of phenytoin serum concentrations into the therapeutic range reduces the frequency of major but not minor seizures. It seems unlikely that the antifolate effect of anticonvulsants is a major factor in the control of epilepsy. Although folic acid and its derivatives act as convulsants when injected into the cerebral cortex of rats or cats, millimolar concentrations of these compounds are required to elicit such effects (Maugiere et al, 1975).

Folate is involved as a cofactor in drug hydroxylation and it is likely that the effect of folate treatment in reducing serum phenytoin concentrations is due to an increased metabolism of the drug. Kutt et al (1966) have previously demonstrated accelerated metabolism of phenytoin to HPPH during folic acid treatment in a patient with phenytoin intoxication. This effect of folate on phenytoin metabolism offers a mechanism whereby administration of a second drug may indirectly increase serum levels of another drug, rather than by competitive inhibition of metabolism. For example, an effect of phenobarbitone in increasing phenytoin serum concentrations (which is suggested to be a possibility in Chapter 4) may be due to a lower serum folate concentration in patients receiving combined therapy, although it seems likely that this would happen only if the patient was receiving a low dose of phenytoin. Also, the non-linear relationship between phenytoin dose and serum concentration may at least partly be due to a lowering of the serum folate concentration as the phenytoin dose is increased.

Brain Amine Metabolism

It has been reported that 5-methyltetrahydrofolate may be an active methyl donor for biogenic amines such as dopamine and serotonin (Laduron, 1972; Banerjee and Snyder, 1974) and this raised the possibility that the effect of anticonvulsants in causing lowered serum and tissue concentrations of folate may in turn cause altered monoamine metabolism. There is some evidence from animal studies that anticonvulsants may produce changes in monoamine metabolism (Bonnycastle et al, 1957; Chase et al, 1969) and this may in part be related to their effects (Meyer and Frey, 1973). Other workers (Reynolds et al, 1975) have found a negative correlation in a group of treated epileptic patients between CSF folate and 5-HIAA or HVA, the metabolites of serotonin and dopamine. In a study of the effect of folic acid in five drug treated epileptics, Hunter et al (1971)

found a slight but not significant fall in HVA though there was a significant drop in HVA in 11 psychiatric patients.

In the present study there were no significant effects on CSF levels of HVA and 5-HIAA when either folic acid or 5-methyltetrahydrofolate were administered. Also, there were no correlations between CSF concentrations of folate and HVA or 5-HIAA in the group of epileptic patients studied, in conflict with previous results, (Reynolds et al, 1975). HVA and 5-HIAA concentrations were lower in epileptic patients as compared to normal controls, whereas a previous report described raised levels especially in patients with high levels of serum anticonvulsants (Chadwick et al, 1975). No correlation was found in the present study between serum anticonvulsant concentrations and the monoamine metabolites in CSF. Other workers, in a study of children with epilepsy, found reduced CSF concentrations of HVA and HIAA afterprobenicid loading, with no relationship to serum anticonvulsant concentrations (Shaywitz et al, 1975); a number of investigations have noted reduced base-line concentrations of one or both of HVA and 5-HIAA in patients with epilepsy (Barolin and Hornykiewicz, 1967; Dubowitz and Rogers, 1969; Papeschi et al, 1972; Garelis and Sourkes, 1973). Shaywitz et al (1975) suggested that the altered brain amine metabolism is related to epilepsy rather than to anticonvulsant medication. Chadwick et al (1975) did not find any difference in CSF amine metabolite levels between neurological controls and untreated epileptics but found that treatment with high serum concentrations of phenytoin and phenobarbitone tended to raise CSF levels of HVA and 5-HIAA, the latter to a significant degree.

A hypothesis which might explain these conflicting results is that there is an abnormality in amine metabolism associated with epilepsy which tends to lower CSF concentrations of HVA and 5-HIAA, and this tends to be reversed by anticonvulsant administration. That Chadwick et al, (1975) did not find lowered concentrations of

HVA and HIAA even in untreated epileptics could be explained by the fact that the patients investigated were out-patients who were presumably suffering from fewer seizures than the in-patients who were investigated in the present study. However, if this was the case one might expect to find a relationship between the lowered levels of HVA and 5-HIAA and the seizure frequency but no relationship was found in the patients examined in the present study. It should also be taken into consideration that these patients, apart from suffering from more seizures than an out-patient epileptic population would be expected to, were also in most cases suffering from some degree of mental deficiency usually associated with brain damage. Thus this in-patient population might not be directly comparable with other groups of patients and there might be a greater degree of abnormality in amine metabolism than is usual.

SUMMARY

1. Serum folate concentrations were measured in 111 patients receiving anticonvulsant therapy. Most patients showed concentrations below the normal range. There was no significant difference in folate concentrations between patients receiving phenytoin and primidone and between patients receiving single or multiple drug therapy, but patients receiving phenobarbitone alone showed higher levels.
2. Folate concentrations in CSF in 43 patients in whom lumbar punctures were performed were also low. There was a significant correlation ($p < 0.01$) between serum and CSF concentrations.
3. Folate was administered, either as folic acid or 5-formyltetrahydrofolate to 19 patients showing low serum and CSF folate concentrations. Both groups of patients showed increases in serum and CSF folate concentrations but there was no effect on seizure control.

4. Serum concentrations in 14 of these patients who were receiving phenytoin showed a significant fall ($p < 0.01$) when folate was administered.
5. Patients treated with anticonvulsants show lowered CSF concentrations of HVA and HIAA but there was no relationship with folate concentrations and folate administration had no effect on levels of these metabolites.
6. It is concluded that there is no relationship between the antifolate effect of anticonvulsants and their therapeutic action or brain amine metabolism. Effects of folate administration in worsening seizure control may be explained by lowered serum concentrations of phenytoin.

CHAPTER 12

THE LIVER ENZYME-INDUCING EFFECTS OF ANTICONVULSANTS

INTRODUCTION

In Section 1 of this thesis it was shown that serum concentrations for a number of anticonvulsants show wide variations between different patients. This was suggested to be frequently due to variations in the rate of metabolism of the drugs, particularly due to variations in the level of enzyme induction. Most anticonvulsant drugs are metabolised by the P_{450} enzyme system of the liver. The basis for induction is an increase of liver size and weight with proliferation of endoplasmic reticulum and increase in enzyme activity per mg of microsomal protein; this can be demonstrated by an increase in cytochrome P_{450} content. The K_m is usually not greatly altered by induction but the V_{max} may become increased manifold (Conney, 1967). If enzyme induction could be measured it might be possible to predict more accurately for a given patient the serum concentration obtained with a given dose of drug.

Direct proof of liver microsomal enzyme induction depends on showing that administration of an inducing agent results in an increase of enzyme activity. Enzyme levels can be assessed directly in liver tissue but liver biopsy is rarely justified for this purpose alone. Indirect evidence comes from the finding of a shortening in plasma half-lives and lowered steady state concentrations in the plasma of drugs metabolised by these enzymes. But the disappearance rate of test drugs such as antipyrine does not necessarily reflect the total activity of drug-hydroxylating enzyme in patients on multiple drug therapy because these drugs may compete with the test drug for the same metabolising enzyme system. Genetic differences in human drug metabolism also complicates the interpretation of drug disappearance rates (Vessell et al, 1969, 1971).

Recently, increases in the activity of the enzyme γ -glutamyl transpeptidase (γ GT) in human serum after administration of phenobarbitone and phenytoin have been reported (Rosalki et al, 1971; Whitfield et al, 1973). Hepatic GT is located primarily in the microsomal fraction (Szewczuk, 1966) and administration of phenobarbitone to rats is accompanied by an increased activity (Ideo et al, 1971). It is likely that the effect of a drug on serum γ GT levels may be related to the drug's ability to promote hepatic enzyme induction.

In this Chapter the measurement of serum levels of γ GT in epileptic patients has been used to assess the relative enzyme-inducing properties of anticonvulsant drugs and to quantify the induction produced by multiple drug therapy. This may allow the prediction of the stimulatory effect on metabolism of a drug due to induction either by itself or by other drugs that the patient is receiving. Also, some of the biochemical effects of anticonvulsants may be due to their enzyme-inducing properties, and attempts have been made to correlate serum γ GT levels with serum concentrations of calcium and triglycerides. In Chapter 11 it was suggested that the effect of anticonvulsants in lowering serum folate concentration may be related to an induction of folate metabolism; in this Chapter the relationship between γ GT and folate levels is described.

PATIENTS AND METHODS

Serum levels of γ GT were measured in 40 male and 38 female epileptic inpatients and outpatients receiving anticonvulsant therapy, their ages ranged from 17 to 71 years (mean 35 years). Twenty five patients were receiving phenytoin, six were receiving phenobarbitone, ten were receiving primdone, two were receiving sodium valproate and the remainder were receiving various combinations of these anticonvulsant drugs. Thirteen patients were also receiving carbamazepine. In twelve of these patients γ GT levels were measured on a second occasion, after being on therapy with sodium valproate. Serum levels of alkaline phosphatase, Ca^{++} and triglyceride were measured in 24 of the patients.

In addition, in this Chapter results for serum concentrations of PEMA and primidone and of clonazepam in patients described in Chapters 5 - 7 have been further analysed and interpreted. Results for folate serum concentrations in patients described in the previous Chapter are also analysed here in terms of the level of enzyme induction; further folate concentrations were measured in 24 patients in whom drug doses had been increased or decreased, so that folate concentrations have been measured in a total of 111 patients on 135 dose regimes. 41 of these were patients in whom γ GT levels were measured.

RESULTS

The γ GT levels in six patients receiving phenobarbitone alone were significantly lower ($p < 0.05$, t-test, comparing the logarithms of the γ GT levels) than in patients receiving phenytoin or primidone as a single drug; also 14 patients receiving combined therapy with phenytoin and primidone showed higher levels ($p < 0.01$) than 22 patients receiving phenytoin alone. The serum levels of γ GT for patients with varying anticonvulsant therapies are given in Table 8.1. On the basis of the levels obtained in patients on single drug therapy, correlating the γ GT levels with the drug doses the patients were receiving, it was possible to quantify the effect of a given dose of a drug in raising γ GT levels. 'Drug Units' were allocated as follows : 50mg of phenytoin equals 1 unit; 60mg of phenobarbitone equals 1.5 units; 250mg of primidone equals 1.5 units. The combined doses of anticonvulsant drugs in patients on multiple drug therapy can then be described in terms of their ability to increase γ GT levels by calculating the Drug Units received. Fig 12.1 shows a plot of serum γ GT levels against Drug Units for 78 patients. A linear plot is obtained when the logarithms of the γ GT levels are plotted against Drug Units, there being significance difference in γ GT levels between male and female patients; the equation of the line is

$$\log \gamma\text{GT} = 1.32 + 0.0567 \text{ Drug Units.}$$

DRUG THERAPY	No of PATIENTS	MEAN SERUM γ GT	SD	RANGE
Phenytoin	25	62	50	10-189
Phenobarbitone	6	28	10	12- 44
Primidone	10	65	41	13-164
Phenytoin & Phenobarbitone	20	62	22	17-101
Phenytoin & Primidone	14	93	40	42-164
Phenobarbitone & Primidone	1	48	-	-
Sodium Valproate	2	15	-	9- 21

Table 12.1 Serum levels of γ GT in 78 patients receiving anticonvulsant therapy

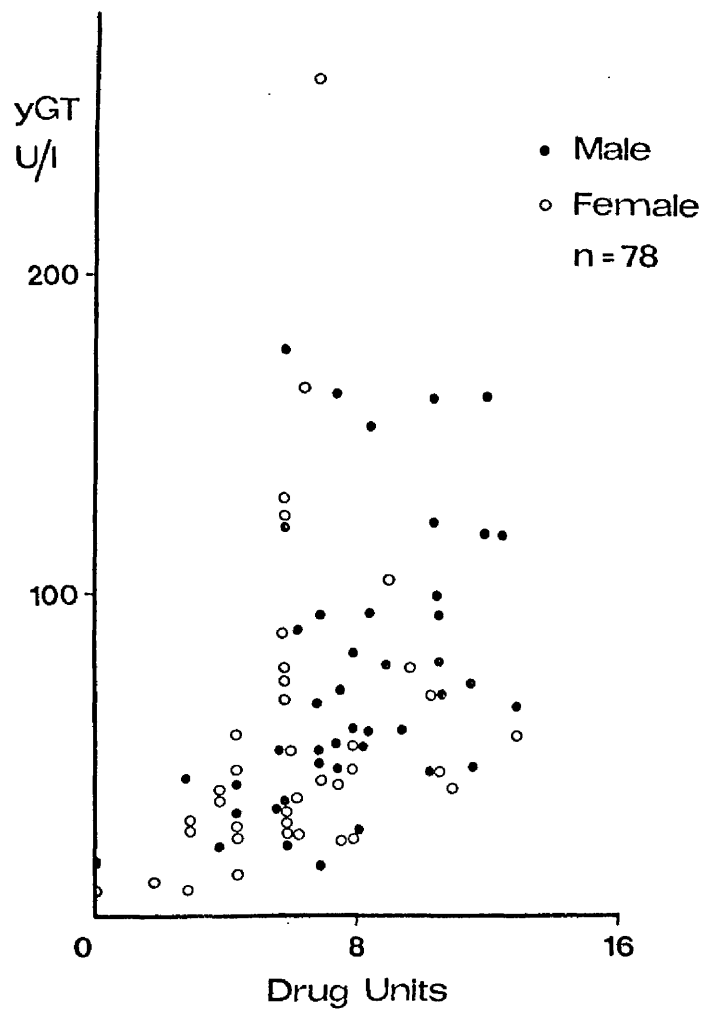


Fig 12.1 Relationship between the serum γ GT levels and the 'Drug Units' that 78 epileptic patients on anticonvulsant therapy were receiving

Administration of sodium valproate to twelve patients produced no change in the γ GT levels. The mean level before treatment was 95.9 U/l and after treatment was 85.2 U/l - there was no significant difference between the means.

Serum Calcium and Alkaline Phosphatase

Serum calcium and alkaline phosphatase were measured in 24 patients. The mean serum calcium concentration was 2.34 ± 0.11 SD mmol/l (normal range 2.12 - 2.62 mmol/l); only one patient showed a level below the normal range. The mean serum alkaline phosphatase level was 34.2 ± 10.5 SD U/l (normal range 12 - 30 U/l); ¹³ patients showed levels above the normal range. There was a significant negative correlation ($r = 0.477$, $p < 0.05$) between the serum calcium and alkaline phosphatase levels.

There was no obvious relationship between the raised alkaline phosphatase levels and the drugs received. Fifteen patients receiving phenytoin (with or without other drugs) had a mean alkaline phosphatase level of 36.1 U/l and nine patients receiving other drugs only had a mean of 31.0 U/l; there was no significant difference between these means. There was no correlation between alkaline phosphatase levels and γ GT levels. There was also no significant correlation between calcium concentrations and the Drug Units received, although there was a possible inverse relationship ($r = 0.201$). However, there was a significant positive correlation ($r = 0.454$, $p < 0.05$) between the logarithm of the γ GT level and the serum calcium level; the equation of the line was $\text{serum calcium} = 2.02 + 0.170 \log \gamma\text{GT}$. In fact, the one patient showing abnormally low serum calcium had the lowest γ GT level (17 U/l).

Serum Triglyceride

Serum triglyceride levels were measured after an overnight fast in the same 24 patients as those above. The levels ranged from 0.77 to 2.62 mmol/l with a mean of 1.66 ± 0.54 SD mmol/l. There was no correlation between triglyceride levels and levels of γ GT.

Serum Folate

Serum folate concentrations were measured in 41 of the patients in whom γ GT levels were measured and the results are shown graphically in Fig 12.2. The serum folate concentrations appear to decrease initially as the γ GT levels increase but the concentrations then level off as shown. Plotting the logarithm of the folate concentration against the logarithm of the γ GT level, a negative straight line correlation was found which only just failed to reach significance at the 0.05 level ($r = 0.297$); the equation of the line was $\log \text{ folate concentration} = 0.58 - 0.135 \log \gamma \text{GT level}$. A significant negative correlation was found when $\log \text{ folate concentration}$ was plotted against Drug Units for 111 patients (in 24 of whom folate concentrations were measured on two different drug regimes) - the equation of the line was $\log \text{ folate concentration} = 0.54 - 0.0179 \text{ Drug Units}$ ($r = 0.283$, $p < 0.001$).

DISCUSSION

The data presented here suggest that both phenytoin and primidone treated subjects show a more severely raised GT level than those treated with phenobarbitone. This is in line with findings by other workers (Rundle and Sudell, 1973). It might, in fact, be expected that the patients receiving primidone would show higher γ GT levels than the patients receiving phenobarbitone since the former had a higher mean serum concentration of derived phenobarbitone, but it may be that primidone itself has an effect since even with doses producing equivalent serum levels of phenobarbitone there appeared to be a greater elevation of γ GT levels, 1000mg of primidone produces a mean serum level of phenobarbitone approximately equal to that produced by 180mg of phenobarbitone itself - the former dose is equal to 6 Drug Units whereas the latter is equal to 4.5 Drug Units. This provides an explanation of the results described in Chapter 3 for serum concentrations of phenytoin in patients receiving phenytoin alone and in patients receiving combined therapy with phenobarbitone or primidone. Patients receiving

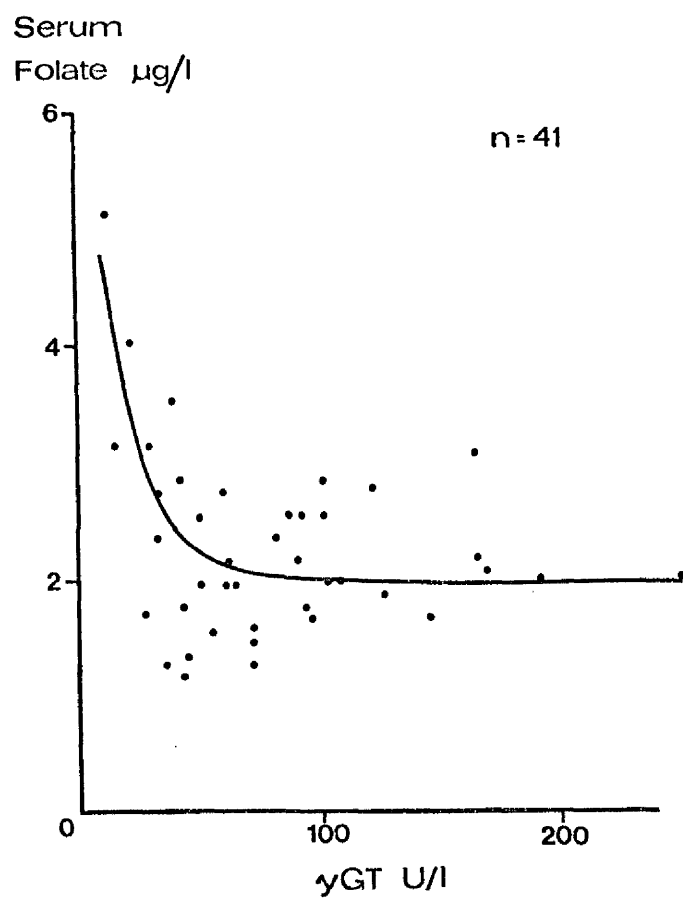


Fig 12.2 Relationship between serum folate and γGT levels in 41 patients on anticonvulsant therapy

primidone appeared to show reduced serum concentrations of phenytoin but this did not occur with phenobarbitone; it is likely that this is due to a higher level of enzyme induction and hence increased phenytoin metabolism in patients receiving primidone.

Patients treated with phenytoin plus primidone showed significantly higher γ GT levels than patients treated with phenytoin alone and thus the effects of these drugs appear to be additive, which suggests that it is reasonable to describe the combined dose of drugs in a patient on multiple therapy according to the potential effect on GT levels and hence presumably their enzyme-inducing properties. The Drug Units ascribed in this study to doses of phenytoin, phenobarbitone and primidone are similar to those ascribed by Richens and Rowe (1970).

The low γ GT levels in the two patients receiving sodium valproate and the lack of elevation in patients on other anticonvulsants who had the drug added to their therapy, suggest that sodium valproate is not an enzyme inducer. This is in line with a study in rats (Jordan et al, 1976) in which there was found to be no effect of sodium valproate administration in rats on levels of cytochrome P₄₅₀ and other liver microsomal enzymes. Thus it is unlikely that the effects of sodium valproate in causing higher serum levels of phenobarbitone and lower serum levels of phenytoin (see Chapter 7) are due to an increase or decrease in the level of induction.

In Chapter 5 it is suggested that phenytoin may induce the metabolism of primidone to PEMA and also that primidone may induce its own metabolism. This is in line with the enzyme-inducing properties of these drugs as measured by their effect on γ GT levels. Fig 12.3 shows a plot of the PEMA/Primidone ratio against the Drug Units received for the patients described in Chapter 5. There is found to be a significant correlation ($r = 0.403$, $p < 0.01$) between the PEMA:primidone ratio and the Drug Units for the 40 patients described in Chapter 5 in whom PEMA concentrations were measured. The Drug Unit concept may be

useful in predicting the serum concentrations of primidone and PEMA obtained in patients on multiple drug therapy.

Similarly, the use of Drug Units may be valuable in predicting serum levels of clonazepam obtained in patients on multiple drug therapy. In Chapters 6 and 7 it was found that patients receiving other drugs generally showed lower clonazepam serum concentrations. Fig 12.4 shows a plot of serum clonazepam concentrations against Drug Units for patients on 3mg clonazepam daily and patients on 6mg clonazepam daily who were described in Chapters 6 and 7 respectively. There is found to be a significant correlation ($p < 0.01$) between the serum concentrations and the Drug Units for both groups of patients. An individual patient who showed good therapeutic response to clonazepam, described in Chapter 7, had a high serum concentration of the drug, although he was receiving fairly high doses of phenytoin and phenobarbitone. This patient showed a normal level of γ GT, suggesting that there was no enzyme induction. Thus in some patients the calculation of Drug Units may not accurately predict enzyme-inducing effects and it may be desirable rather to measure serum GT in the individual patients.

Calcium Metabolism

Osteomalacia has been recognised as a complication of anticonvulsant therapy (Dent et al, 1970) and has been attributed to induction of the liver microsomal enzymes which convert cholecalciferol and 25-hydroxycholecalciferol into inactive metabolites (Hahn et al, 1972). Low serum calcium and raised levels of alkaline phosphatase in treated patients have been described (Richens and Rowe, 1970; Hunter et al, 1971) as have low levels of 25-hydroxycholecalciferol (Hahn et al, 1975). If enzyme induction was responsible for the disordered calcium metabolism, one might expect to find a negative correlation between serum γ GT activity and the serum calcium level.

Richens and Rowe (1970) produced a system for scoring anticonvulsant drugs - one unit was allowed for every 50mg of phenytoin or 30mg phenobarbitone and 1.5 units for every

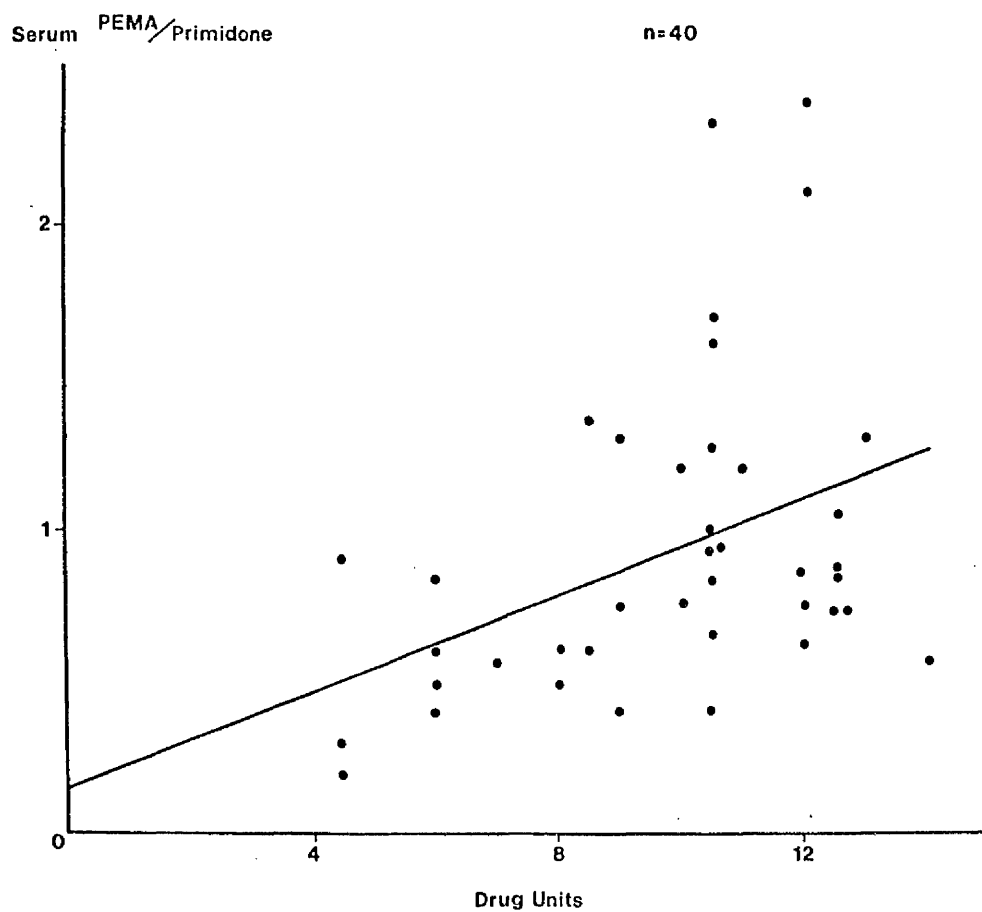


Fig 12.3 Relationship between the serum PEMA/primidone ratio and the 'Drug Units' that 40 patients on primidone therapy were receiving. A significant positive relationship ($p < 0.01$) is shown

Serum Clonazepam
nmol/l

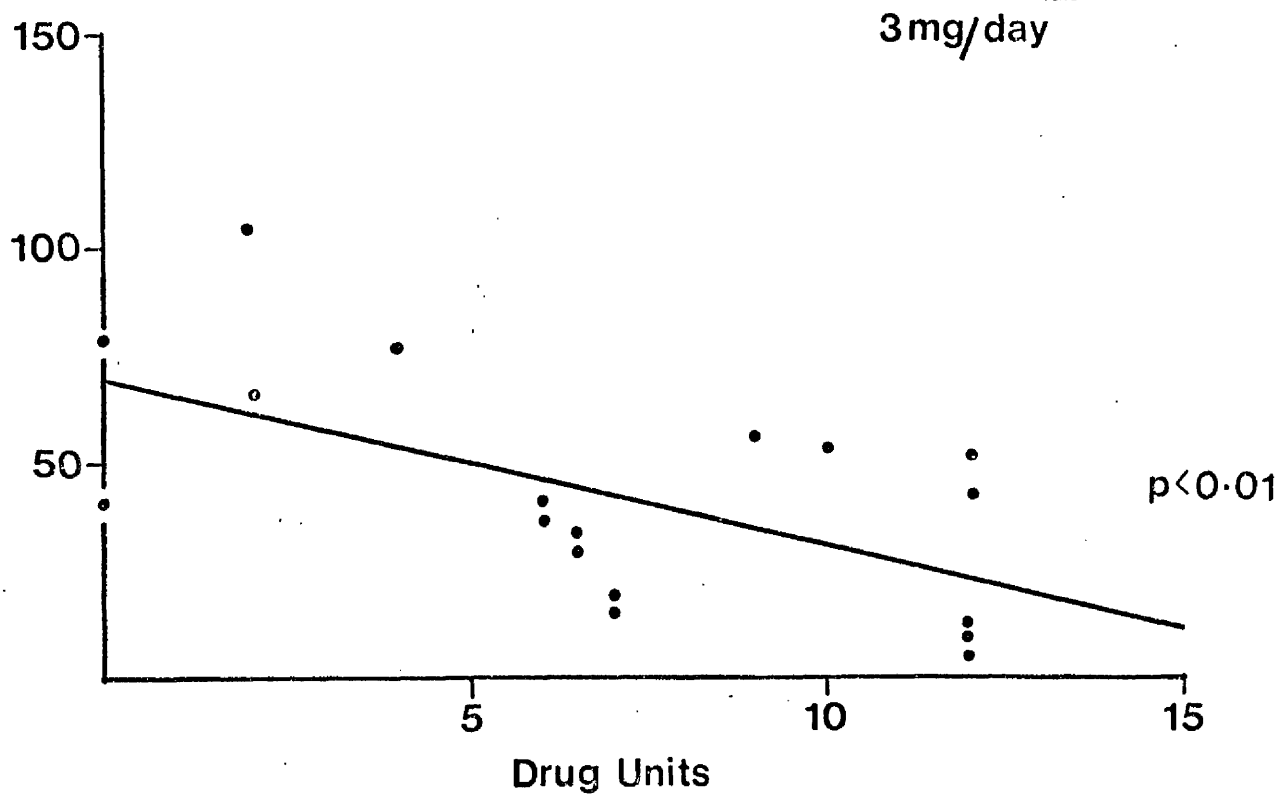
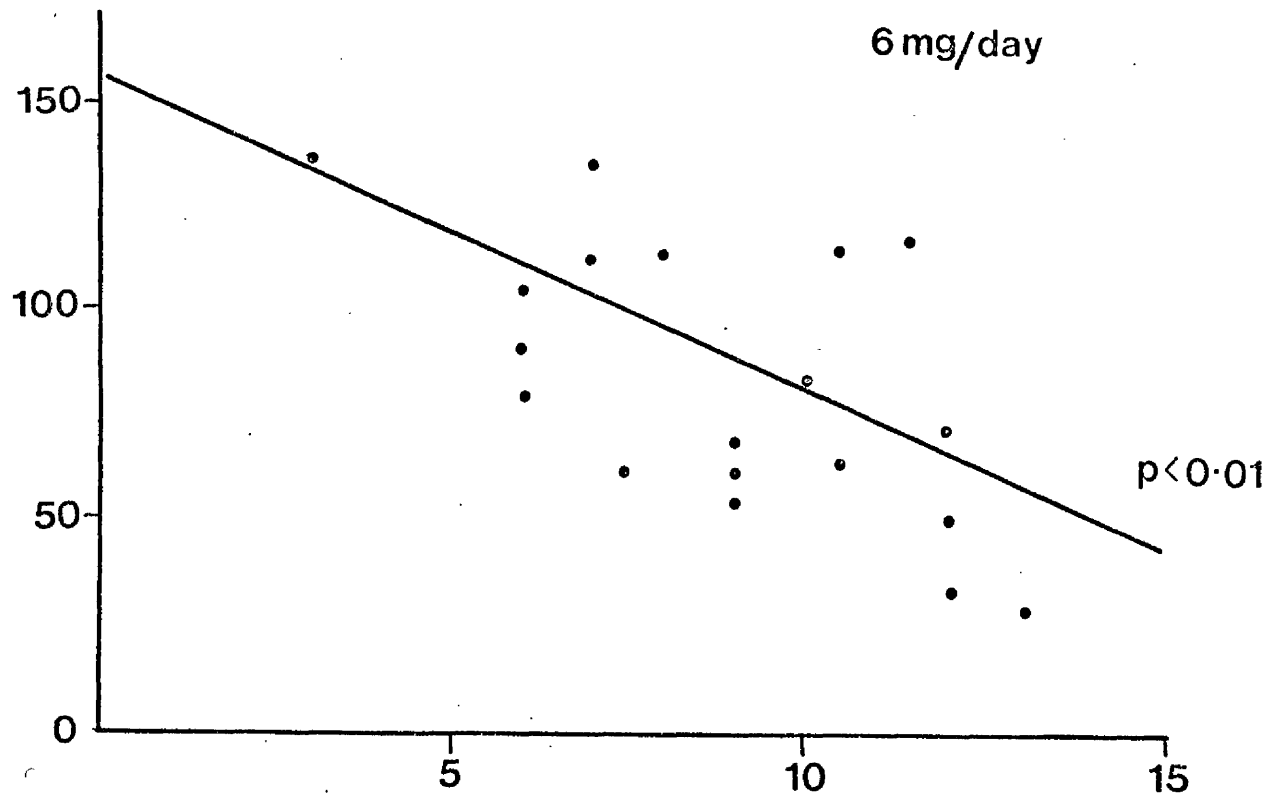


Fig 12.4

Relationship between serum clonazepam concentrations and the 'Drug Units' that the patients were receiving

250mg of primidone - and found a significant inverse correlation between these Drug Units and the serum calcium level. The system for scoring Drug Units devised from the present study is similar, but only 0.75 units is allowed for every 30mg of phenobarbitone. Using this system, the negative correlation although non-significant ($r = 0.201$) was in fact higher than in the larger group of patients investigated in the previous study ($r = 0.17$). Thus the present study would appear to provide further evidence that the disturbance of calcium metabolism in epileptics is related to an effect of anticonvulsants on liver enzyme induction being an increased breakdown of vitamin D. However, in the present study a significant positive relationship was found between the γ GT levels and the serum calcium, which is the opposite of what would be expected. This would suggest that, while the lowered serum calcium may be related to the total doses of drugs that the patient is receiving, it is not due to an enzyme induction effect; it is possible that enzyme induction may in fact raise serum calcium levels.

Serum Triglycerides

A correlation between the concentration of serum triglycerides and of serum γ GT within the general population has been reported (Martin et al, 1975). It was suggested that induction of a microsomal enzyme on the biosynthetic pathway for triglycerides may cause elevated serum triglyceride. In addition, there has been a report that administration of oral contraceptives may produce raised levels of both serum triglyceride and γ GT (Martin et al, 1976) and a study of a few patients on anticonvulsant therapy found raised serum triglyceride and γ GT (Annoni et al, 1976). Patients in the present study did not show serum triglyceride levels outwith the normal range and there was no correlation with serum and GT concentrations. This accords with studies by other workers of the effects of administration of phenobarbitone to normal subjects, there being no significant change in total triglyceride (Durrington et al, 1976; Ohnhaus et al, 1977). Another study has shown an

increase in serum triglyceride after the start of phenytoin therapy but this change was a temporary one (Reunanen and Sotaniemi, 1976). The results in the present study suggest that there is no permanent change in serum triglyceride in patients on chronic anticonvulsant therapy.

Serum Folate

The finding of an association between the serum γ GT level and the level of serum folate is evidence that the folate deficiency in patients on anticonvulsant therapy may be related to the induction of hepatic drug-metabolising enzymes. Similarly Maxwell et al (1972) have found a linear relationship between D-glucaric acid excretion when plotted logarithmically against the serum folate in a group of children. It appears that there is a relationship between serum folate and the drug dose but only at low concentrations of enzyme-inducing drugs; the folate tends not to fall below a certain point and levels off with high drug doses.

However, it remains uncertain whether the lowered folate is really due to induction of folate metabolism in the liver. There may be no direct casual relationship between the level of enzyme induction and the serum folate concentration. However, studies in rats (Spray and Burns, 1972; Williams and Spray, 1976) have shown that administration of phenobarbitone or phenytoin significantly increased the liver activities of glutamate formimino-transferase and methylene tetrafolate dehydrogenase, enzymes involved in folate metabolism.

SUMMARY

1. Serum levels of γ GT were assayed in 78 patients receiving anticonvulsant therapy as a measure of the level of enzyme induction. The γ GT levels appeared to be related to the number and doses of drugs that the patient was receiving and it was possible to quantify the enzyme-inducing effect of a given dose of a drug in terms of 'Drug Units'.

2. Serum calcium and alkaline phosphatase levels were measured in 24 patients. A number of patients showed raised alkaline phosphatase levels and there was a significant negative correlation ($p < 0.05$) between alkaline phosphatase and calcium levels although only one patient showed an abnormally low serum calcium level. The serum calcium levels appeared to be inversely related to the Drug Units but there was a significant positive correlation ($p < 0.05$) between serum calcium and the GT level.
3. Serum triglyceride levels were measured in 24 patients. They were all in the normal range and there was no correlation with γ GT levels.
4. The use of Drug Units may be valuable in predicting serum concentrations of primidone or clonazepam in patients on multiple drug therapy. Significant correlations were found between the PEMA;primidone ratio ($p < 0.01$) or clonazepam serum concentrations ($p < 0.01$) and the Drug Units that a patient was receiving.
5. A significant correlation was found between serum folate concentrations and the Drug Units that the patients were receiving ($p < 0.001$). Lowered serum folate in patients receiving anticonvulsant therapy may be due to induction of folate metabolism.
6. It is concluded that the rate of metabolism of clonazepam and primidone and the lowered folate levels in patients on anticonvulsant therapy may be related to enzyme. However, there was no apparent effect of enzyme induction on serum calcium or triglyceride levels.

CHAPTER 13

THE EFFECTS OF ANTICONVULSANTS ON SERUM IMMUNOGLOBULIN LEVELS

INTRODUCTION

Abnormally low levels of serum IgA have been found in patients receiving phenytoin (Slavin et al, 1974; Aarli and Tönder, 1975; Sorrell and Forbes, 1975; Seager et al, 1975; Fontana, 1976). In some patients also low IgG has been reported both in serum (MacKinney and Booker, 1972; Aarli, 1975) and in CSF (Fossan, 1976). It remains unclear whether there is a specific effect of phenytoin, or whether other anticonvulsants also reduce levels of IgA and possibly other immunoglobulins. This Chapter describes immunoglobulin levels in patients receiving differed anticonvulsant regimes; IgA levels have been compared in patients receiving phenytoin and patients receiving other drugs only. The possible mechanisms for a lowering of IgA levels are discussed and particularly the relationship between immunoglobulin metabolism and serum folate concentrations. The effect of folate administration on immunoglobulin levels in the patients described in Chapter 11 is described.

PATIENTS AND METHODS

The patients studied were 83 epileptic inpatients who had been on anticonvulsant therapy for at least one year. Of these, 56 were receiving phenytoin and 27 were receiving other anticonvulsants only. All the patients were receiving at least one of the anticonvulsants phenytoin, phenobarbitone or primidone; in addition some patients were receiving other drugs - carbamazepine (15), sodium valproate (8), clonazepam (2), diazepam (1), sulthiame (1).

Serum immunoglobulin (IgG, IgA and IgM) levels were measured in all patients on one occasion and in most patients on two occasions. In addition, serum concentrations of anticonvulsants and of folate were measured in these patients.

Serum immunoglobulin levels were measured in the 19 patients described in Chapter 11 who received therapy with folic acid or 5-methyltetrahydrofolate. Levels were measured before and one month after the start of folate therapy.

RESULTS

The 100% mean normal values for IgG, IgA and IgM in the laboratory in which the serum immunoglobulin measurements were carried out are IgG 947mg/100ml, IgA 248mg/100ml, IgM 94mg/100ml and the normal range is regarded as 50-175% MNA (Hobbs, 1971) with a log-normal distribution. The serum immunoglobulin levels for 82 patients receiving anticonvulsant therapy are summarised in Table 13.1 and the IgA levels are shown in Fig 13.1. The serum levels of IgG and IgM were generally within the normal range (although five patients showed somewhat lowered IgM levels) but thirteen of the patients receiving phenytoin (23%) had serum levels of IgA below 50% MNA. Only one of the patients not receiving phenytoin had a slight reduction, but there was no significant difference in the mean IgA level between patients receiving phenytoin and patients receiving only phenobarbitone or primidone ($t = 1.94$, $p < 0.1$, comparing the log values of the IgA levels). There was no correlation between serum IgA levels and levels of IgG or IgM.

The serum anticonvulsant levels in these patients are summarised in Table 13.2. The serum phenobarbitone levels in 4 patients with low IgA were significantly higher than in 24 patients with normal IgA ($p < 0.05$, t -test) who were also receiving combined therapy with phenytoin and phenobarbitone. In addition, in patients receiving primidone and phenytoin, there was a significantly lower ($p < 0.05$) serum primidone concentration in seven patients who were IgA deficient compared to 16 patients with normal IgA, although the phenobarbitone concentrations in the two

DRUG THERAPY	NO OF PATIENTS	MEAN SERUM FOLATE	SERUM IgA		SERUM IgG		SERUM IgM	
			MEAN	SD	MEAN	SD	MEAN	SD
Phenytoin	5	2.1	220	160	1325	490	147	18
Phenobarbitone	10	3.9	250	50	1125	250	104	48
Primidone	14	2.7	320	140	1170	290	108	59
Phenytoin + Phenobarbitone	29	2.5	290	160	1110	270	100	34
Phenytoin + Primidone	22	2.5	215	135	1140	445	90	39
Phenobarbitone + Primidone	3	3.0	380	145	1150	25	75	5

Table 13.1
Serum Immunoglobulin Levels in 83 Epileptic Inpatients
Receiving Anticonvulsant Therapy

Table 13.1

DRUGS	NO	LOW SERUM IgA	NO	PATIENTS ON PHENYTOIN NORMAL SERUM IgA	NO	ON OTHER DRUGS NORMAL SERUM IgA
Phenytoin	13	43±24 SD	43	42±26 SD	-	-
Phenobarbitone	4	179±63 SD	24	119±46 SD	10	112±52 SD
Primidone Phenobarbitone	7	17± 9 SD 123±42 SD	16	33±19 SD 120±58SD	15	23± 9 SD 118±60 SD

Table 13.2

Serum Anticonvulsant Levels in $\mu\text{mol/l}$ in the Patients in whom
Serum IgA Was Measured

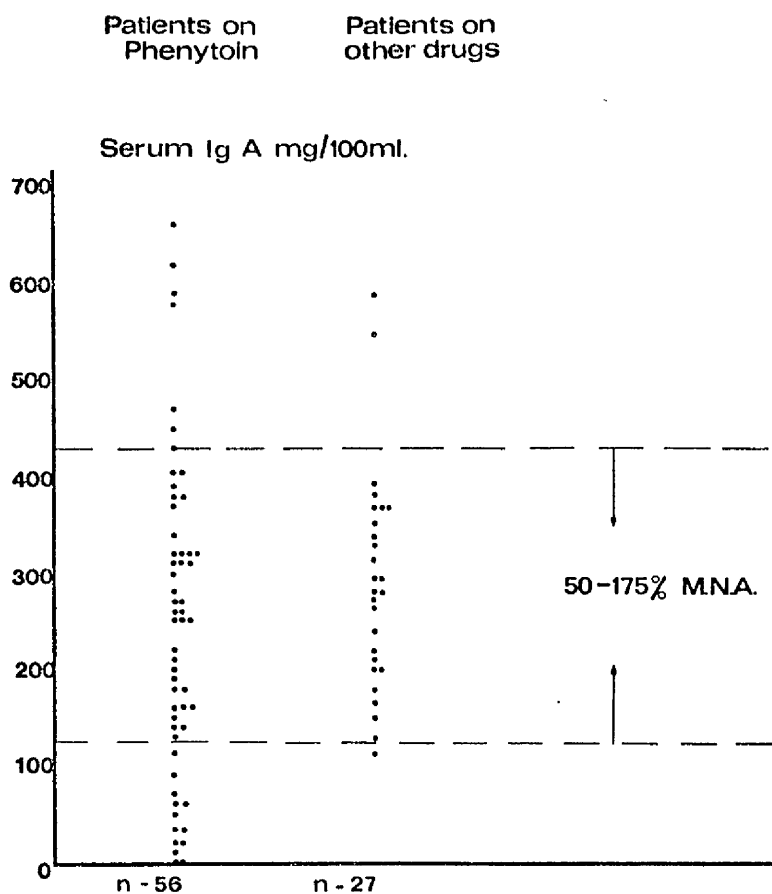


Fig 13.1 Serum IgA levels in 56 patients on phenytoin therapy and 27 patients receiving other anticonvulsants only

groups were the same. There was no apparent relationship between the reduced IgA levels and serum concentrations of phenytoin.

Patients receiving phenytoin had lower serum folate concentrations than patients receiving other anticonvulsants only, but the higher occurrence of abnormally low serum levels of IgA did not appear to be related to this. There was no correlation between reduced serum folate concentrations and low IgA levels; in fact, there was a negative trend between serum folate and IgA levels. There was also a negative trend which just reached significance between serum folate and IgG levels ($r = 0.237$, $p < 0.05$, correlating the log values of the folate and IgG levels).

In order to investigate the relationship between the immunoglobulin levels and the number and doses of drugs that the patients were receiving, immunoglobulin levels were correlated with 'Drug Units' (the method for calculating Drug Units is described in the previous Chapter). There were negative trends between Drug Units and immunoglobulin levels for IgG, IgA and IgM but this did not reach significance in any instance.

Serum immunoglobulin levels were measured in 19 patients who received folate therapy. There was a significant fall in levels of both IgA and IgG ($p < 0.01$, Wilcoxon signed-ranks test) but not of IgM after folate therapy.

DISCUSSION

The incidence of IgA deficiency in the normal adult population is one in 500-700 individuals (Hobbs, 1971). In the present study, abnormally low serum IgA was found in 23% of patients receiving phenytoin, which correlates well with the findings of other workers (Slavin et al, 1974; Sorrell and Forbes, 1975; Aarli, 1976). The higher incidence of low IgA levels in patients on phenytoin relative to patients on other drugs would suggest that there is a specific effect of phenytoin on IgA metabolism. However, there was no significant difference between the mean serum IgA levels for patients receiving phenytoin and patients

receiving other drugs; also, it should be noted that the patients receiving phenytoin were generally receiving higher combined doses on anticonvulsant drugs, being treated with phenytoin and phenobarbitone or primidone in combination. If there is a specific effect of phenytoin on IgA levels, then a negative relationship between serum phenytoin and IgA levels might be expected; this was not found.

The reduction of IgA levels may be a consequence of reduced production or of increased breakdown. It is possible that anticonvulsants might cause increased breakdown of IgA (and possibly of other immunoglobulins) by their effects on liver growth and enzyme induction. Little is known about immunoglobulin catabolism but it is known that 30% of IgG is broken down in the liver and high doses of corticosteroids can increase breakdown.

In patients in the present study receiving primidone, there were significantly higher phenobarbitone concentrations and lower primidone concentrations in patients with low IgA levels; a positive relationship between serum primidone and IgA levels has also been reported by Slavin et al (1974). The increased phenobarbitone:primidone ratio in patients with low IgA levels could be explained by an increased metabolism of primidone in these patients (see Chapter 5) and this would be in line with the hypothesis that there is higher enzyme induction in these patients, which increases the metabolism of IgA in the liver. However, no correlation was found between the IgA levels and the enzyme induction as measured by increased serum levels of γ GT. In addition, although there was a negative trend, there was no correlation between IgA (or IgG and IgM) levels and the combined doses of enzyme - inducing drugs as expressed in 'Drug Units'.

It was noted that there were negative trends towards lower levels of IgA and IgG (significant in the case of IgG) in patients with higher serum concentrations of folate. This is other than might be expected since patients show low concentrations of folate on high doses of anticonvulsants (see Chapter 11) and patients on phenytoin showed lower folate concentrations than patients receiving other anticonvulsants only. When folate was administered to patients

on anticonvulsant therapy, significant reductions in levels of IgA and IgG were observed. The most likely explanation for these findings is that folate is involved in immunoglobulin catabolism and that the low folate concentrations obtained in patients on anticonvulsant therapy inhibit metabolism. It is thus hypothesised that there are two opposing effects of anticonvulsants on immunoglobulin metabolism - they directly increase breakdown by their enzyme-inducing effects but indirectly also decrease their breakdown via their effects in lowering folate concentrations. It may be that there are also more widespread effects of anticonvulsants on serum protein metabolism. For example there has been a report of decreased serum haemopexin and C_3 concentrations and increased serum caeruloplasmin after phenytoin therapy (Seager, 1977); it is possible that these conflicting effects may reflect similar effects of phenytoin on protein catabolism to those suggested for immunoglobulins, although it is also possible that phenytoin may induce protein synthesis by the liver.

SUMMARY

1. Serum immunoglobulin levels were measured in 83 patients receiving anticonvulsants. 23% of patients receiving phenytoin showed serum IgA levels below the normal range.
2. There was no apparent relationship between serum phenytoin levels and levels of IgA but there were significantly lower serum primidone levels in patients who were IgA deficient. It is suggested that this may reflect an increased rate of metabolism, both of IgA and of primidone in these patients.
3. There was a significant negative relationship ($p < 0.05$) in these patients between serum folate and IgG levels and administration of folate to the 19 patients described in Chapter 11 significantly

reduced serum levels of IgG and IgA ($p < 0.01$).
It is possible that the low serum folate concentrations in patients on anticonvulsant therapy may inhibit immunoglobulin breakdown.

APPENDIX

METHODS USED IN THIS THESIS

In Chapter 2 brief summaries of the methods used in this thesis for measurement of anticonvulsants were given. More extensive descriptions are given here of these methods. In addition, this Appendix includes details of methods used for measuring protein binding of drugs and of methods for assay of various enzymes and metabolites in body fluids.

Extraction of Phenytoin, Phenobarbitone, Primidone, HPPH and PEMA from Biological Fluids

The method was based on that of Goudie and Burnett (1973) and was basically the same for plasma, whole blood, urine or CSF samples. HPPH was present in measureable quantities only in urine.

Materials

1. 1mg/ml solutions in MeOH of the above drugs and metabolites stored at 4°C
2. Internal standard. Stock solution 1mg/ml p-tolyl phenytoin in MeOH
3. Dichloromethane (Anala r)
4. Cyclohexane (Anala r)
5. 10mM NaOH
6. 1M HCL
7. 9cm phase-separating paper (Whatman)

Standardisation

Working standards

1. Drugs. Pipette 1ml of each of the relevant stock solutions into a volumetric flask and make up to 20ml with 10mM NaOH (50 µg/ml)
2. Internal Standard. Dilute 1ml of stock solution with 4ml of 10mM NaOH

Procedure

1. Preparation of Standard Curve. Make up the following dilutions with 10mM NaOH of the working standard : 0,5,10,20,30,40,50 µg/ml of drug. Pipette 1ml of each of these dilutions into test tubes and add 1ml of horse serum
2. Preparation of Specimens. Add 1ml specimen and 1ml 10mM NaOH to test tubes
3. Extraction. To standards and tests add 0.1ml internal standard and 0.1ml 1M HCl. Add 5ml cyclohexane and mix for 5 minutes by shaking. Centrifuge briefly and discard upper cyclohexane phase.

Add 10ml dichloromethane and mix for 5 minutes. Remove aqueous phase using phase separating paper (which has been previously washed with dichloromethane). Evaporate the extract to dryness at 50° in water bath under nitrogen. The residues can be stored in fridge for up to one week prior to chromatography.

Gas Chromatographic Analysis of Phenytoin, Phenobarbitone
Primidone and HPPH

The method is based on that of Goudie and Burnett (1973) but using a gas chromatograph with flame ionisation detectors rather than nitrogen detectors. The method has been extended to include assay of HPPH. A Pye Unicam 104 gas chromatograph equipped with dual flame ionisation detectors and temperature programming was used. The drugs were chromatographed on 3-foot long coiled glass columns packed with 3% OV-1 on chromsorb mesh WCHP (Pye Unicam)

1. Add 0.1ml of 25mM trimethylphenyl-ammonium hydroxide (TMPAH) to dried extract in conical tube immediately before chromatograph. Mix on vortex mixer for 15 seconds and inject 2 μ l into GLC.
2. Gas chromatography conditions :
Column oven programmed 1 min at 200°C then 10°C/min to 275°C
Detector oven 300°C
Injection point heater 275°C
Nitrogen carrier flow rate 60ml/min
Attenuation 1×10^3
The peaks obtained are shown in the accompanying figure. Peak heights due to the drugs are measured in relation to the internal standard (p-toly-phenytoin)

Note : Administration of carbamazepine was found to interfere slightly with the assay of primidone

Gas Chromatographic Analysis of PEMA

The method is based on that of Baumel et al (1972). The gas chromatograph and column used were as for the previous assay.

1. Add 0.2ml of N,N - Dimethylformamide to 1ml of bis (trimethylsilyl) acetamide. Add 20 μ l of this to residue (5 minutes before injection) and mix on vortex for 15 seconds. Inject 2 μ l.
2. Gas chromatography conditions :
Column oven programmed 2 minutes at 190°C, then 15°C min to 240°C. Detector oven 300°C.
Nitrogen flow rate 60ml/min. Attenuation 1×10^3 .
The peaks obtained for primidone and PEMA are shown in the accompanying figure. The peak due to PEMA is measured in relation to the peak due to primidone, the concentration of the latter being measured by the method above.

Note : Administration of ethotoin was found to interfere markedly with this assay.

Assay of Sodium Valproate by Gas Chromatography

The method used was similar to that of Chard (1976). A Pye Unicam 104 gas chromatograph with flame ionisation detectors was used as in the previous assays but there was no requirement for temperature programming.

Standardisation

1. Sodium valproate standard solution - 1mg/ml in horse serum
2. Internal standard solution - 1mg/ml cyclohexane carboxylic acid in H_2SO_4
3. Preparation of standard curve. Make up the following dilutions with horse serum of the sodium valproate standard solution - 0,20,50,100,150,200 $\mu g/ml$

Extraction

Pipette 100 μl serum (for both standards and tests), 100 μl internal standard solution and 100 μl dichloromethane into a small glass tube. Mix on vortex for 30 seconds and centrifuge (1 minuted at 2000 rpm) to give a clear lower organic layer. Inject 2 μl into GLC.

Gas Chromatography

Column 3ft x 2mm packed with 2% W/11 on Chromasorb W(HP) 80-100 mesh (Perkin-Elmer Limited). Oven temperature 150°C. Detector temperature 250°C. Nitrogen flow rate 30ml/min. Attenuation 2×10^3

Radioimmunoassay of Phenytoin

This was used to measure phenytoin concentrations in saliva, described in Chapter 9. The method was a specific double-antibody radioimmunoassay using an antiserum raised against phenytoin-valerate-BSA (Paxton et al, 1976) and an iodinated tracer (Paxton et al, 1977). 20 μ l of sample (serum, saliva or CSF) is incubated with an appropriate dilution of phenytoin antiserum, donkey anti-rabbit precipitating serum (Wellcome) and trace amounts of ^{125}I -phenytoin for 1 hour at 37°C . After centrifugation of the incubate at 4°C for 30 minutes at 2500g the supernatant is aspirated and the precipitate (containing the antibody bound ^{125}I -phenytoin) counted for 10-20 seconds on a counter.

The detection limit of the assay was approximately 0.4 nmol/l and there was a good correlation with the gas chromatographic method ($r = 0.95$ with gradient = 1.13) used in this study in a double-blind comparison on 200 serum samples obtained from epileptic patients over a six month period (Paxton et al, 1977).

Plasma Protein Binding of Phenytoin and Phenobarbitone

Heparinised blood was obtained from patients receiving one or more of the anticonvulsants phenytoin, phenobarbitone or primidone. The plasma was removed and small concentrations of ^3H -labelled phenytoin and ^{14}C -labelled phenobarbitone (see following section) were added. Ultrafiltration through cellulose tubing was carried out by the method of Lunde et al (1970) in cellophane dialysis tubing (Visking) with a flat diameter of 6mm. The tubing was first soaked in isotonic saline for 15 minutes and the excess water squeezed out. One end was carefully double knotted and 2ml of labelled plasma was added to the bag, which was then suspended in a 10ml glass centrifuge tube. The bags were centrifuged twice at 800xg for 10 and 20 minutes respectively. The filtrate obtained after the first centrifugation, corresponding to the water content of the bag, was discarded. The drug content in 50 μl of the second filtrate was determined by radioassay as described in the following section. This filtrate was routinely screened for protein by observing the precipitation upon adding one drop of concentrated perchloric acid. All studies were carried out at room temperature.

Equilibrium Dialysis

Equilibrium dialysis was also carried out using 6mm cellophane dialysis tubing. 1ml of labelled plasma was inserted into a bag, which was knotted at both ends. The bag was then placed in a small stoppered plastic tube containing 2ml of isotonic phosphate buffer (pH 7.4) such that the top of the buffer solution in the tube just covered the top of the plasma in the bag. The tube was stoppered and placed on a rotating mixer. Equilibration was allowed to take place for 16 hours at a constant temperature. The drug content in 100 μl of the dialysate was determined by radioimmunoassay.

Measurement of Protein Binding using Ultrafiltration Cell

In Chapter 8 a study to determine the effects of sodium valproate on protein binding of phenytoin and phenobarbitone is described. An 'Amicon' Model 12 ultrafiltration cell was used (volume 10ml), the plasma being filtered through a 'Diaflo' UM-10 membrane. Rotary stirring is provided by means of a magnet and the driving force for ultrafiltration is nitrogen gas.

The cell was initially charged with 10ml of plasma. Small volumes of radioactively labelled phenytoin and phenobarbitone were added to give a concentration of 20 $\mu\text{g/ml}$ of each. The cell was immediately pressurised and the filtrate collected. Fractions collected at approximately 10, 20 and 30 minutes were taken for analysis by scintillation counting. Immediately after the last fraction was collected, pressurisation was removed and sodium valproate was added to the plasma in the cell to give a concentration of 100 $\mu\text{g/ml}$. The cell was re-pressurised and further fractions for analysis obtained at 40, 50 and 60 minutes. The total filtrate volume obtained throughout the experiment was less than 10% (ie 1ml) of the cell charge. The experiment was carried out at room temperature.

It is theoretically possible using this method to determine the binding kinetics of drugs to proteins. However, it was found that the rates of binding of phenytoin and phenobarbitone to plasma proteins were too rapid for this to be accomplished.

Radioassay of Phenytoin and Phenobarbitone

This assay was used in studies described in Chapters 8 and 10 involving measurement of drug binding in plasma or uptake by RBC's. The radioactively labelled drugs were added to plasma or whole blood in vitro.

Materials

1. ^3H -labelled phenytoin (5 Ci/mmol) (Radiochemical Centre, Amersham) 25 μCi dissolved in 10ml ethanol.
2. ^{14}C -labelled phenobarbitone (20 Ci/mmol) (Radiochemical Centre) 50 μCi dissolved in 10ml ethanol.
3. NE 260 scintillation fluid (Nuclear Enterprises Ltd)
4. 0.9% saline.
5. Glacial acetic acid.
6. 70% hydrogen peroxide (H_2O_2).

Method

1. 1ml of stock solutions of each labelled drugs are combined and made up to 5ml with isotonic saline to make working solution.
2. For measurement of uptake by RBC, 0.1ml of working solution is added to 4ml whole blood. Both 0.1ml of plasma and 0.1ml of RBC (resuspended to original volume in isotonic saline) are taken for counting.
3. For measurement of protein binding by ultrafiltration of equilibrium dialysis, 0.1ml of working solution is added to 2.5ml plasma, 0.05 ml of ultrafiltrate or 0.1ml of dialysate are taken for counting.
4. For labelled plasma ultrafiltrate or dialysate, add to 5ml of scintillation fluid and count for 5 minutes.
5. For labelled RBC, add 0.1ml glacial acetic acid and 0.1ml H_2O_2 to 0.1ml RBC in scintillation vial to decolourise RBC. Heat open vials at 70° for 30 minutes. Add 5ml scintillation fluid and count for 5 minutes.
6. ^3H and ^{14}C are both counted in Channel 1 while ^{14}C alone is counted in Channel 2. A ^{14}C standard is used to calculate the ratio of ^{14}C counts in Channel 1 to ^{14}C counts in Channel 2. Channel 1-Channel 2 x ratio gives the ^3H counts.

Enzymes and Metabolites in Body Fluids

Folate

Chapter 11 involves a study of folate concentrations in serum and CSF. A modification of the L.casei method (Herbert, 1966) was used. This was carried out in the Department of Haematology of the Southern General Hospital, Glasgow.

HVA and 5-HIAA

Measurements of these amine metabolites in CSF are described in Chapter 11. They were estimated spectrophotofluorimetrically by the method of Ashcroft et al (1968). The fluorimeter used was an Aminco-BowmanSPF125 fitted with an off-axis ellipsoidal condensing system.

Immunoglobulins

Serum immunoglobulin (IgG, IgA, IgM) concentrations described in Chapter 13 were measured by nephelometry using the technicon AIP system. These measurements were performed in the Department of Haematology.

Serum Albumin

Albumin concentrations described in Chapter 8 were measured colorimetrically by a method based on the binding of the dye bromcresol green (Doumas and Biggs, 1972).

The following enzymes and metabolites were determined using Boehringer Test Combination Kits.

γ Glutamyl Transferase

Chapter 12 involves studies of serum levels of γ GT, which were measured colorimetrically by a method based on hydrolysis of the substrate.

Alkaline Phosphatase

Alkaline phosphatase levels in serum, described in Chapter 12 were measured colorimetrically by a method based on the hydrolysis of the substrate p-nitrophenylphosphate.

Triglycerides

Serum triglyceride concentrations, also described in Chapter 12 were measured spectrophotometrically by a method involving saponification and then assay of the free glycerol by the NAD produced in its reaction with glycerokinase.

Creatinine

Chapters 4B and 5 include studies of concentrations of drugs and metabolites in 24-hour urine specimens. Completeness of collection was checked roughly by measuring creatine production. Creatinine in urine was measured colorimetrically by a method involving the reaction with picric acid.

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